University of Alberta

Effect of feeding formaldehyde and heat treated canola seed on milk yield and milk composition in early-lactation cows.

by

Sandra Michelle Tymchuk

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science

in

Nutrition and Metabolism

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August 11, 1997

DEDICATION

To my husband Corey and my parents,
Mary and John Tymchuk.
Corey and Mom: Thank you for all your
help, support and encouragement.
Dad: Thanks for watching over me.
All my thanks and love,
Sam

ABSTRACT

Four early-lactation Holstein cows were used in a 4 x 4 Latin square design to determine the effect of dietary fat on ruminal digestion, nutrient delivery to the intestine and milk composition. Diets were control (no added fat) and control plus either untreated, formaldehyde or heat treated canola seed. Formaldehyde treatment reduced ruminal disappearance and effective degradability of dry matter, protein and lipid. Canola seed did not influence intake, rumen fermentation, duodenal nitrogen fractions or milk yield. Formaldehyde treatment increased the proportion of unsaturated fatty acids (linoleic (76%), linolenic (123.5%)) and decreased palmitic acid (17.9%). Overall, the concentration of long-chain fatty acids increased at the expense of short- and medium-chain fatty acids as a result of formaldehyde treatment. Untreated and heat treated canola seed produced similar effects but they were less pronounced than observed for formaldehyde treatment.

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LIST OF SYMBOLS, NOMENCLATURE AND ABBREVIATIONS

SYMBOLS

β	Beta
Δ	Delta
κ	Kappa
%	Percent
ω	Omega
°C	Degrees Celsius

NOMENCLATURE

$C_{4:0}$	Butryic acid
$C_{6:0}$	Caporic acid
$C_{8:0}$	Caprylic acid
$C_{10:0}$	Capric acid
$C_{12:0}$	Lauric acid
$C_{14:0}$	Myristic acid
$C_{16:0}$	Palmitic acid
$C_{16:1}$	Palmitoleic acid
$C_{18:0}$	Stearic acid
$C_{18:1}$	Oleic acid
$C_{18:2}$	Linoleic acid
$C_{18:3}$	Linolenic acid
HCl	Hydrochloric acid
NH_3N	Ammonia nitrogen

ABBREVIATIONS

ACC	Acetyl CoA carboxylase
ADF	Acid detergent fibre
ADIN	Acid detergent insoluble nitrogen
ADOM	Organic matter apparently digested in the rumen
BCS	Body condition score
CP	Crude protein
Cr	Chromium
DAPA	Diaminopimelic acid
DIM	Days in milk
DIP	Rumen degradable protein
d	day
dl	decilitre
DM	Dry matter
FAS	Fatty acid synthtase
FFA	Free fatty acids
g	gram
GC	Gas chromatography

GLC	**Gas liquid chromatography**
GLM	**General linear models**
h	**hour**
HDL	**High density lipoproteins**
IDL	**Intermediate density lipoproteins**
kg	**kilogram**
LDL	**Low density lipoproteins**
LpL	**Lipoprotein lipase**
lsmeans	**Least square means**
min	**minute**
mg	**milligram**
ml	**millilitre**
mmol	**millimole**
N	**Nitrogen**
NDF	**Nuetral detergent fibre**
NPN	**Non-protein nitrogen**
OM	**Organic matter**
SD	**Standard deviation**
SEM	**Standard error of the mean**
SNK	**Student Newman-Keuls**
TMR	**Total mixed ration**
μg	**microgram**
UIP	**Rumen undegradable protein**
VFA	**Volatile fatty acids**
VLDL	**Very low density lipoproteins**

CHAPTER 1 - INTRODUCTION

Effect of Feeding Formaldehyde and Heat Treated Canola Seed on Milk Yield and Composition in Early-Lactation Cows.

Introduction

1.1. Fatty Acid Nomenclature

Triglycerides consist of three fatty acids attached to a molecule of glycerol. The fatty acids present in milk fat account for approximately 94% by weight of milk fat and are present as triglycerides (Brumby and Welch, 1978). The physical and chemical characteristics of fatty acids are determined by the length of the carbon chain in the fatty acid and the number and position of double bonds present along the carbon chain. Naturally occurring fatty acids consist mainly of unbranched, even numbered carbon chains which can be either saturated, monounsaturated or polyunsaturated.

Fatty acids are chains of carbon with a carboxyl group at one end that can combine with another molecule and an unreactive methyl group at the other end (Enig, 1993). The three fatty acids of a triglyceride can be a combination of saturated or unsaturated fatty acids. The terms saturated or unsaturated refer to the number of double bonds along the carbon chain. Enig (1993) states a saturated fatty acid has adequate hydrogens and is chemically stable, whereas, an unsaturated fatty acid is missing adequate hydrogens and is chemically unstable. Unsaturated fatty acids contain double bonds, whereas, saturated fatty acids do not contain double bonds. For example, $C_{18:0}$ has a carbon length of 18 and it has no double bonds (saturated) as indicated by "0". A designation of $C_{18:3}$ indicates a carbon length of 18 and three unsaturated bonds. The position of the double bond along the carbon chain is also important. A method of designating the position of the double bond is to count the carbons from the active end of the chain. For example, linoleic acid is designated as $C_{18:3\omega3}$ which is an 18 carbon fatty acid with three double bonds with the first double bond beginning at the third carbon atom from the methyl carbon atom at the end of the fatty acid.

Monounsaturated fatty acids contain only one double bond. A monounsaturated fatty acid, therefore, is missing two hydrogens and in place of these hydrogens the adjacent carbons "double" bond to each other (Enig, 1993). The major monounsaturated fatty acid in milk is oleic acid ($C_{18:1}$). The polyunsaturated fatty acids contain two or more double bonds. A polyunsaturated fatty acid, therefore, is missing four to six hydrogens (Enig, 1993). Yaqoob and Calder (1993) describe the three families of unsaturated fatty acids which are named from the position in the fat structure of the double bonds: (1) omega-9, (2) omega-6 and (3) omega-3. The omega-9 unsaturated fatty acids can be synthesized by animals, whereas, the omega-6 and omega-3 polyunsaturated fatty acids are not. These unsaturated fatty acids are not metabolically interconvertible. The major polyunsaturated fatty acid in milk is linoleic acid ($C_{18:2\ n\text{-}6}$). Examples of omega-3, omega-6 and omega-9 fatty acids are linolenic acid, linoleic acid and oleic acid, respectively. For each double bond present, two hydrogen atoms are required to saturate the fatty acid molecule. Unsaturated fatty acids can be hydrogenated by rumen microbes to become saturated fatty acids. The main saturated fatty acid in milk is palmitic acid ($C_{16:0}$).

Unsaturated fatty acids can also be described by their *cis* or *trans* configurations. Enig (1993) provides a description of *cis* and *trans* fatty acids. Fatty acids which have the hydrogens attached to the carbon on the same side of the double bond have *cis* configurations and those with the hydrogens on the opposite side of the double bond have *trans* configurations. The double bonds which naturally occur in plant oil fatty acids are typically curved with a *cis* configuration, therefore, the fatty acids cannot pack into a crystal form at normal room temperatures. These unsaturated fatty acids produce a liquid fat or an oil at room temperature. The saturated fatty acids have a straight configuration and most of these fatty acids can pack into a solid crystal at room temperature. When unsaturated fatty acids are altered by partial hydrogenation to straighten the chains in order to have some of the physical packing properties of saturated fatty acids, their *cis* double bond has been changed to a *trans* double bond. This results in the fatty acid being changed from a mostly unsaturated oil into a solid fat. The *trans* fatty acids are the same length and weight as the original *cis* fatty acids from which they were derived but their curvature is different. Isomers is the term

which is used to describe the relationship between the hydrogens and carbons to each other. Hydrogens attached to the carbons the same side of the double bond are called *cis* isomers. Hydrogens attached to the carbons on the opposite sides of the double bond are called *trans* isomers. Isomerization is the term used to describe the change from the *cis* configuration to the *trans* configuration. The dominant isomers are Δ (delta)-8, Δ-9, Δ-10, Δ-11, Δ-12 and Δ-13. The symbol Δ (delta) marks the carbon on the chain where the double bond occurs. The dominant *trans* isomer is Δ-11, *trans*-vaccenic acid. Natural fats are generally *cis* and their double bonds include Δ-9 and/or Δ-12 and/or Δ-15.

Another classification of fatty acids is the volatile fatty acids (VFA). The VFA are short-chain fatty acids such as acetic, propionic and butyric which contain 2, 3 and 4 carbon fatty atoms, respectively. Volatile fatty acids are the most important microbial fermentation products that are readily absorbed and utilized by the ruminant animal. Ruminant animals derive a major portion of their energy from VFA as well as from glucose and other monosaccharides. Acetate and propionate are the most abundant VFA. The relative amounts of these VFA vary with time after eating, diet, rumen pH and the microbial contents in the rumen.

Fatty acids, therefore, can be classified by several criteria: (1) carbon length which varies from two to 24, (2) volatile fatty acids, (3) unsaturated or saturated (4) mono and polyunsaturated (5) *cis* and *trans* configurations or (6) omega fatty acids (Enig, 1993, Yaqoob and Calder, 1993, Miller, 1979).

1.2. From the Diet to the Dairy Cow

Dietary fat usually constitutes less than 3% of ruminant diets and arises from forage, grains or seeds which are rich in linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) (Chilliard, 1993, Palmquist and Jenkins, 1980, Miller, 1979). A typical ruminant diet consists of forage crops such as legumes or grass and concentrates such as barley or corn. Neither forage or cereals contain high proportions of lipid and the lipid present consists mainly of esterified polyunsaturated fatty acids (Christie, 1981). The main lipid component of forages is $C_{18:3}$, whereas, the main lipid component of concentrates (cereals) is $C_{18:2}$. Lipids are used in

ruminant diets for three reasons: (1) the high caloric value of lipids can be useful in overcoming limitations in energy supplies of high yielding animals (concentrated source of energy), (2) to alter fatty acid composition of meat or milk and (3) some fat sources are low priced feedstuffs and can be used as a replacement for grain energy (Chilliard, 1993, Wu et al., 1991, Storry, 1981). Fat has an energy value of about 2.25 times that of carbohydrates and proteins and could potentially increase dietary energy density (Palmquist and Jenkins, 1980). During early-lactation dairy cows are in negative energy balance because dietary energy intake is less than energy output for maintenance and milk production and rely extensively on the mobilization of long-chain fatty acids from adipose tissue to satisfy their energy requirements for maintenance and lactation. The synthesis and mobilization of fat reserves from adipose tissue is considered to be less efficient than with direct utilization of dietary fat sources (Miller, 1979). Palmquist and Jenkins (1980) indicate fat can adversely affect rumen fermentation and nutrient digestibility, free fatty acids can depress rumen microbial growth and the degree of saturation can influence rumen function and nutrient digestibility. With these factors in mind there are limitations on the inclusion levels of fat in the diet. Generally up to 5% fat can be added to the diet (7-8% total dietary fat). Exceeding these limits may cause problems with rumen fermentation and digestibility.

A relatively new area of research involves feeding protected fat supplements. Greater amounts of protected fat can be fed in comparison to unprotected fat sources without causing detrimental effects on rumen function due to the protected lipid being relatively inert in the rumen. Protection of a dietary lipid results in the fat being protected against rumen fermentation but susceptible to hydrolytic digestion in the intestine. The prime objective of feeding a protected lipid source, other than increasing the energy density of the diet, is to change milk fat composition. Feeding unprotected fat in the diet has resulted in changes in milk fat composition (Wu et al., 1994, Elliott et al., 1994, 1993), however, these changes are more pronounced when protected fat supplements are utilized (Khorasani and Kennelly, 1996, Ashes et al., 1992). The changes which occur are a decrease in the short-chain ($C_{4:0}$ - $C_{12:0}$) and medium-chain ($C_{14:0}$ - $C_{16:0}$) fatty acids and an increase in the long-chain fatty acids (C_{18}). More specifically, the objective of feeding a protected lipid source is to increase the

concentration of unsaturated fatty acids while decreasing the concentration of saturated fatty acids.

The characteristic feature of ruminant milk fat is the high proportion of short- and medium-chain fatty acids and the low proportion of polyunsaturated fatty acids (Brumby and Welch, 1978). Modifying the fatty acid composition of milk by using protected lipid sources may eventually lead to the creation of a "designer" milk. With continued research in the area of protected lipid sources, it could be possible to move in the direction of an ideal nutritional milk as described by O'Donnell (1989) and Grummer (1991). The Wisconsin Marketing Board looked towards industry and academia for advice to define the ideal milk fat composition. The recommendation given by the Wisconsin Marketing Board 1988 Roundtable was a milk fat which contains up to 10% polyunsaturated fatty acids, 8% saturated fatty acids and 82% monounsaturated fatty acids. Typical milk fat consists of 5% polyunsaturated fatty acids, 70% saturated fatty acids and 25% monounsaturated fatty acids (Kennelly, 1996b, Khorasani and Kennelly, 1996, Ashes et al. 1995). The ideal nutritional milk fat suggested by the Roundtable is considerably different than typical milk fat produced by the dairy cow. Kennelly and Khorasani (1992) have stated that research indicates that the balance of monounsaturated, polyunsaturated and saturated fatty acids rather than the absolute value is the most important factor from a human health perspective. The concept of a designer milk can be a target for the future, however, the changes required may not need to be as extreme as those in the ideal milk fat suggested by Wisconsin Marketing Board 1988 Roundtable.

1.3. Lipid Metabolism

1.3.1. Fatty Acid Synthesis

Enser (1984) describes fatty acid synthesis in animals, both ruminants and non-ruminants. Enser (1984) states that fatty acids are synthesized from any body component that yields a two-carbon acetyl unit during its metabolism. The principle mechanism of fatty acid synthesis in many animal tissues involves two basic reactions (Moore and Christie, 1981). The first reaction involves the carboxylation of acetyl CoA to malonyl CoA and is catalyzed

by acetyl CoA carboxylase (ACC). The second reaction is catalyzed by a group of enzymes know as fatty acid synthase (FAS) which results in chain elongation (addition of two carbon units) up to C_{16} and sometimes C_{18}. Acetyl CoA carboxylase adds carbon dioxide to the acetyl CoA to yield malonyl CoA. A malonyl group and an acetyl group are transferred from CoA to the FAS complex and are condensed to yield acetoacetyl-S-enzyme and carbon dioxide is released. The sequential reduction of the ketoacyl group is carried out by the enzyme system, dehydration of the hydroxyacyl group and the reduction of enoyl double bond to yield a saturated fatty acid which is two carbons longer. The cycle is then repeated using malonyl CoA until the fatty acid is released from the enzyme. The length of the fatty acid synthesized depends upon the tissue in which the synthesis takes place (Enser, 1984). Moore and Christie (1981) indicate that fatty acid synthesis in the mammary tissue of ruminants occurs predominately from the malonyl pathway, however, some $C_{4:0}$ can be synthesized by an alternative pathway possibly involving the reversal of β-oxidation. The review of Moore and Christie (1981) also indicated that carbon atoms 1 and 2 of $C_{4:0}$ are derived from malonyl CoA and carbon atoms 3 and 4 from acetyl CoA, whereas, $C_{6:0}$ and $C_{8:0}$ are synthesized by successive addition of C_2 units derived from malonyl CoA. The synthesis of long-chain fatty acids from acetyl CoA and malonyl CoA involves a sequence of six reactions for each two carbon addition, the sequence is repeated several times to produce a long-chain fatty acid (Goodridge, 1991).

Schulz (1991) and Goodridge (1991) describe the regulation of fatty acid synthesis. The cellular concentration of malonyl CoA is directly related to the activity of ACC which is hormonally regulated. Short term regulation of ACC involves the phosphorylation and dephosphorylation (covalent modification) of the enzyme and allosteric regulators, citrate and long-chain fatty acid acyl CoA may act synergistically with covalent modification (Goodridge, 1991). Long term regulation can be triggered by the same factors, however, is affected at the level of transcription, translation or post-translational modification. Increased concentration of citric acid activates ACC as well as acts as a source of acetyl CoA while a high concentration of long-chain acyl CoA acts as an inhibitor. In order for fatty acid synthesis to be inhibited while they are being mobilized for degradation, the enzyme is inactivated by

cyclic-AMP-dependent protein kinase through phosphorylation cascade. Citrate plays a lesser role in the ruminant mammary gland as the pyruvate/malate shuttle system is not active. Acetyl CoA carboxylase, therefore, is considered to be the rate limiting enzyme in fatty acid synthesis (Enser, 1984, Storry, 1981). Metabolic, hormonal and dietary control regulate the synthesis of monounsaturated fatty acids.

1.3.2. Ruminal Digestion and Biohydrogenation of Lipid

Rumen microorganisms are able to synthesize fatty acids, fat and other lipids which are needed for cell protoplasm. Rumen microbes, therefore, can supply the ruminant animal with its fat needs without the presence of a dietary fat source. The rumen bacteria and protozoa can synthesize fatty acids and fats from carbohydrates and other nutrients from the animal's body tissues. There are, however, essential dietary fatty acids which ruminants require that cannot be produced by rumen microorganisms. These include $C_{18:2}$, $C_{18:3}$ and arachidonic ($C_{20:4}$) acid. These fatty acids must be obtained from dietary fat sources found in the diet of the ruminant. The key essential fatty acid is $C_{18:2}$ since it can meet the animal's requirement for essential fatty acids. Linolenic and arachidonic acids can partially substitute for $C_{18:2}$ (Miller, 1979).

After feed consumption by the adult ruminant, lipid digestion begins in the reticulorumen. The initial stages of ruminal digestion are characterized by lipolysis, fatty acid hydrogenation and de novo lipid synthesis by rumen microorganisms (Bauchart, 1993). The microorganisms in the rumen possess strong lipase activity which synthesize galactosyl-acylglycerols and triacylglyercols from galactose, glycerol and free fatty acids (FFA). Brumby and Welch (1978) reported that the release of FFA from ingested lipids is important since it affects carbohydrate and cellulose digestion in the rumen. Increased uptake of fats and oils results in a reduced rate of cellulose degradation and thus, less acetate production resulting from microbial metabolism of carbohydrate. Brumby and Welch (1978) have shown that this leads to a reduction in intramammary synthesis of short- and medium-chain fatty acids which may offset an increase in mammary uptake of dietary fatty acids. Galactose and glycerol are metabolized to VFA (mainly acetate and propionate) which can be absorbed from the rumen.

The long-chain unsaturated fatty acids are hydrogenated to saturated and monounsaturated acids which pass in the digesta to the abomasum and then to the small intestine (McDonald and Scott, 1977).

Dietary long-chain saturated fatty acids pass through the rumen unchanged and are absorbed and incorporated into the animal's tissues. Dietary unsaturated fatty acids are subjected to hydrogenation or partial hydrogenation by rumen microorganisms prior to passing into the intestinal tract (Christie, 1981). The initial step in biohydrogenation is an isomerization reaction. Biohydrogenation of $C_{18:2}$ and $C_{18:3}$ initially involves an isomerization reaction that converts the *cis*-12 double bond in the unsaturated fatty acids to a *trans*-11 isomer. Once the *trans*-11 bond is formed the hydrogenation of $C_{18:2}$ occurs and the fatty acid is converted to $C_{18:0}$ (stearic acid). Stearic acid is the principle end product of microbial hydrogenation of $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ fatty acids (Jenkins, 1993). The $C_{18:2}$ and $C_{18:3}$ are, therefore, hydrogenated to $C_{18:0}$ or a number of geometrical (*trans*) and positional isomers of monounsaturated acids ($C_{18:1}$) (Holmes et al., 1984). *Trans* fatty acids are primarily formed from incomplete biohydrogenation of ingested $C_{18:2}$ and $C_{18:3}$ by rumen microorganisms (Ney, 1991). Some of the unchanged $C_{18:2}$ may be converted to $C_{20:4}$ and other longer-chain fatty acids, while the $C_{18:0}$ can be desaturated to $C_{18:1}$ (Christie, 1981).

Hydrogenation of $C_{18:2}$ and $C_{18:3}$ to $C_{18:0}$ requires the presence of several species of bacteria, the products of one serving as substrates of others (Brumby and Welch, 1978). Brumby and Welch (1978) indicate that there has been no evidence to indicate a role for protozoa in the hydrogenation process, however, it has been shown that unsaturated fatty acids have been more effectively hydrogenated when protozoa and bacteria are both present in the rumen. Jenkins (1993) also indicates that branch-chain fatty acids are derived from the utilization of isobutyrate, isovalerate and 2-methylbutyrate. Brumby and Welch (1978) indicate the biosynthesis of branch-chain fatty acids involves the oxidative deamination of valine, leucine or isoleucine to give isobutyric or methylbutyric acids which are utilized in the biosynthesis of iso and anteiso series of fatty acids. The odd-chain fatty acids arise from the use of propionate and valerate as precursors for de novo fat synthesis. The monounsaturated fatty acids that constitute 15-20% of bacterial fatty acids are synthesized by an anaerobic

pathway.

In summary, the major changes carried out in the rumen on dietary lipids are hydrolysis and the biohydrogenation of released fatty acids by the microbial population. This leads to 70-90% reduction of the dietary polyunsaturated fatty acids and their transformation to saturated or *trans* isomers of monounsaturated fatty acids (Chilliard, 1993).

1.3.3. Absorption and Transport of Lipids

After the hydrolysis, interconversion and hydrogenation of fatty acids in the rumen, the long-chain fatty acids enter the small intestine. Storry (1981) and Brumby and Welch (1978) report that small amounts of fatty acids are absorbed from the upper jejunum (pH 2-4) and the remainder are from the lower three quarters of the jejunum (pH 7). The absorption of FFA occurs gradually as the digesta goes through the intestinal tract, with 5% of the total absorption occurring in the duodenum, 20% in the upper jejumun, 25% in the mid and lower jejunum and 50% in the ileum (Bauchart, 1993). Miller (1979) states that any fat which remains intact when the digesta reaches the small intestine is hydrolysed to glycerol and fatty acids under the influence of lipase. The action of microbial lipases in the rumen cause most of the ingested fatty acids entering the duodenum to be in an unesterified form (Brumby and Welch, 1978). The ingested fatty acids which have not been released because of incorporation into microbial lipids or through some other form of protection are acted upon by lipase and phospholipases secreted in pancreatic juice. Brumby and Welch (1978) also state that biliary lecithin is progressively hydrolysed during the course of its passage down the small intestine. Also, both lecithin and lysolecithin as well as phosphatidylethanolamine and bile salts aid in the dissociation of the FFA from particulate matter and their uptake in micellar solution. The fatty acid anion contributes to micelle formation and the amount of fatty acid solubilized increases with pH (Brumby and Welch, 1978).

Moore and Christie (1981) state that prior to absorption, bile constituents facilitate the transfer of the absorbed lipid to a soluble micellar phase. When the absorbed fatty acids enter the enterocytes of the small intestine, they are converted back to their coenzyme A derivatives before being synthesized back into triacyglycerols via the α-glycerophosphate or

monoacylglycerol pathways. After resynthesis, the fatty acids are packaged into lipoproteins which are discharged by reverse pinocytosis. The main lipoprotein responsible for transporting dietary fat from the intestine is the chylomicron, however, the intestine also secretes very low density lipoproteins (VLDL). The lipoproteins are then passed into the lymph system to the venous system by the intestinal and thoracic lymph ducts.

1.3.4. In the Blood Plasma

The next step in dietary fat metabolism is the packaged chylomicrons passing into the blood plasma. In the blood plasma, the fatty acids exist as chylomicrons and FFA. Lipids are hydrophobic and are transported in blood in association with protein in complex macromolecules termed lipoproteins (Brumby and Welch, 1978). The plasma lipoproteins are divided into five major density classes based on their physical properties and chemical composition. The classes include chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), intermediate density lipoproteins (IDL) and high density lipoproteins (HDL), which reflects their relative lipid-protein content (Bauchart, 1993, Brumby and Welch, 1978).

Bauchart (1993) provides a detailed description of lipoproteins. The chylomicrons are the largest and the least dense and formed in the absorptive cells of the intestine. The main role of these triglyceride rich lipoproteins is to transport dietary fatty acids as triglycerides from the small intestine to tissues such as adipose tissue, the mammary gland, muscle and liver. Chylomicrons are rarely seen in bovine plasma as they are absorbed in the lymph in the form of VLDL. Bovine VLDL represent an alternative form of triglyceride transfer in lymphatic and blood vessels from the intestine. Plasma VLDL are generally low in the bovine compared to humans since VLDL represent 0.5% of the total lipoproteins in the dairy cow. Very low density lipoproteins are mainly from the liver in humans, however, there is not a high proportion in ruminants as the liver has limited ability to package and secrete fatty acids. Intermediate density lipoproteins are intermediates in the VLDL-LDL cascade and are generated during the lipolysis of VLDL. Bovine LDL are end products of intravascular degradation of VLDL via LDL and are implicated in the cholesterol distribution

to tissues. Low density lipoproteins represent a minor lipoprotein class (<10% of total lipoproteins). High density lipoproteins are the major plasma lipoproteins (>80% of total lipoproteins) which are synthesized and secreted by the liver and the small intestine. These are the main particles implicated in a reverse cholesterol transport system that returns excess cholesterol from peripheral cells to the liver for bile excretion and resynthesis of new VLDL particles (Bauchart, 1993, Brumby and Welch, 1978).

1.3.5. To the Liver, Muscle, Adipose Tissue and the Mammary Gland

Chylomicrons and FFA from the blood plasma can be metabolized by the liver, muscle tissue, adipose tissue or the mammary gland. In the muscle, chylomicrons and lipoproteins are converted to FFA which can form triglycerides, phospholipids or are oxidized to carbon dioxide. Free fatty acids in the muscle can be mobilized from and incorporated back into the blood plasma to be utilized by other tissues. Free fatty acids in the adipose tissue can be synthesized into triglycerides and oxidized to carbon dioxide. Mobilization of the FFA in the adipose tissue to triglycerides in the liver can, therefore, provide a pool of fatty acids for use in milk fat synthesis. As with the muscle tissue, FFA can be mobilized from the adipose tissue and released into the blood plasma to be used elsewhere in the body.

In the liver, the chylomicrons and FFA can be metabolized to carbon dioxide, phospholipids or triglycerides. The triglycerides and the phospholipids can then be mobilized from the liver to form lipoproteins in the blood plasma that can be utilized in the muscle, adipose tissue or the mammary gland. The ruminant liver, however, is a minor site of fatty acid synthesis, therefore, fatty acids must be imported by the liver before metabolism (Veron, 1980). The major fatty acid supply must come from plasma fatty acids or from body stores (ie: adipose tissue). Bovine liver also lacks the ability to convert $C_{18:0}$ to $C_{18:1}$ (St. John et al., 1991). Emery et al. (1992) states the liver has four options for fatty acid disposition: (1) secretion into bile, (2) oxidized completely to carbon dioxide or partially to acetic acid or ketone bodies, (3) stored in the liver or (4) secreted in lipoproteins. Cholesterol, which is synthesized in the liver, is used to form lipoproteins in the blood plasma.

1.3.6. In the Mammary Gland

Chlyomicrons, VLDL and FFA provide substrates for milk fat synthesis. The desaturase activity in the mammary gland converts the saturated fatty acids (ie: $C_{18:0}$) to monounsaturated fatty acids (ie: $C_{18:1}$) which helps to balance the extensive hydrogenation that took place in the rumen (Grummer, 1991). Under normal dietary conditions, $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ acids are considered to be the principle fatty acids present in blood triglycerides and are the fatty acids which are normally taken up by the mammary gland (Moore and Christie, 1981). Dietary modifications which cause changes in the fatty acid composition of the blood triglycerides also cause changes in the composition of the fatty acids which are taken up by the mammary gland (Moore and Christie, 1981).

Fatty acids in milk are derived from two major sources, (1) acetate and β-hydroxybutyrate through de novo synthesis in the mammary gland and (2) long-chain fatty acids derived from triacylglycerols of chylomicrons and VLDL in the blood plasma (circulating blood lipids). The approximate percentages of these milk fat precursors are as follows: (1) acetate 30-45%, (2) β-hydroxybutyrate 8% and (3) triglycerides 40-60% (Beitz and Allen, 1989). Acetate, therefore, is the main precursor of fatty acid synthesis in the mammary gland and several biochemical pathways are involved: (1) carboxylation of acetyl CoA to malonyl CoA followed by a sequential condensation of the malonyl CoA with primer molecules of acetyl CoA to give fatty acids containing up to 16 carbon atoms, (2) direct incorporation of β-hydroxybutyrate as a C_4 molecule which can be elongated by further additions of acetyl CoA and (3) desaturation of $C_{18:0}$ and $C_{16:0}$ (Storry, 1981). In order for acetate, which enters the alveolar cells in the mammary tissue, to be utilized it must be converted into acetyl CoA in the cytosol. Fatty acids, therefore, are synthesized from acetate and β-hydroxybutyrate from the malonyl CoA pathway (Holmes et al., 1984). Synthesis of fatty acids occurs in the cell cytosol where the conversion of acetyl CoA to malonyl CoA by ACC is the rate limiting step (Storry, 1981). The following outline indicates how fatty acids are synthesized from acetate and β-hydroxybutyrate by the malonyl CoA pathway (Moore and Christie, 1981):

(1) Acetyl CoA + CO_2 + ATP – (Acetyl-CoA carboxylase) → Malonyl CoA + ADP + phosphate

(2) Malonyl CoA + S-ACP* – (Malonyl CoA-ACP transferase) → Malonyl-S-ACP + CoA

(3) Acetyl-S-ACP + Malonyl-S-ACP – (β-ketoacyl-ACP-synthetase) → Acetoacetyl-S-ACP + CO_2+ SH-ACP

(4)Acetoacetyl-S-ACP + NADPH + H+ –(β-ketoacyl-ACP-reductase) → β-hydroxybutyryl-S-ACP + NADP+

(5) β-hydroxybutyryl-S-ACP – (β-hydroxyacyl-ACP dehydrase) → Crotonyl-S-ACP + H_2O

(6) Crotonyl-S-ACP + NADPH + H+ –(Crotonyl-ACP reductase) → Butyryl-S-ACP + NADP+

(7) Butyryl-S-ACP + Malonyl-S-ACP –(steps 3 to 6 repeated) → Fatty acyl-S-ACP + CO_2

(8) Fatty acyl-ACP + H_2O–(Fatty acyl deacylase) → Fatty acid

*reduced acyl-carrier protein

The overall equation for the synthesis of $C_{16:0}$ is as follows (Moore and Christie, 1981):

Acetyl-CoA + 7 Malonyl CoA + 14 NADPH → Palmitate (C_{16}) + 7 CO_2 + 14 NADP+ + 8 CoASH.

Moore and Christie (1981) in their review indicated that 50% of butyrate found in milk fat originates from condensation of two C_2 units derived from acetate and 50% from the intact C_4 skeleton of β-hydroxybutyrate. Butyrate provides a C_4 unit which is condensed sequentially with one or more C_2 units derived from acetate to form milk fatty acids from $C_{6:0}$ to $C_{16:0}$. All of the milk fatty acids from $C_{6:0}$ to $C_{12:0}$, most of the $C_{14:0}$ and approximately 60% of the $C_{16:0}$ are synthesized by this process in the mammary gland. The remainder of the $C_{14:0}$ and $C_{16:0}$ and all the C_{18} fatty acids are derived from the constituent fatty acids of the plasma lipids. About half of the four carbon atoms at the methyl end of the fatty acid originate from an intact C_4 unit derived directly from β-hydroxybutyrate and the remaining carbon atoms originate from C_2 units derived from acetate. Acetate is the precursor for the remaining fatty acids synthesized de novo in the mammary gland. Moore and Christie (1981) state that

acetate and β-hydroxybutyrate are the two important and under most conditions, possibly the only sources of carbon for de novo fatty acid synthesis.

In summary short-chain fatty acids ($C_{4:0}$ - $C_{10:0}$) are synthesized from de novo synthesis in the mammary gland primarily from acetate and β-hydroxybutyrate, the long-chain fatty acids ($C_{18:0}$ and greater) are derived from circulating triglycerides in blood plasma and medium-chain fatty acids ($C_{12:0}$ - $C_{16:0}$) result from both routes (Rook and Thomas, 1980).

The 1981 review by Moore and Christie indicates that the ability of the mammary gland to synthesize short- and medium-chain fatty acids depends upon the physiological state of the mammary gland. Kinsella (1975) indicated that 18 days prior to parturition only trace amounts of fatty acids from $C_{4:0}$ to $C_{10:0}$ were synthesized by cow mammary gland tissue. The majority of the fatty acids consisted of $C_{16:0}$, $C_{14:0}$, $C_{18:0}$ and $C_{12:0}$ (60, 30, 5 and 4%, respectively). Seven days before parturition, synthesis of short- and medium-chain fatty acids increased and the synthesis of $C_{16:0}$ and $C_{18:0}$ began to decrease. The changes in the pattern of fatty acid synthesis by mammary tissue continued until the seventh day after parturition when the fatty acids from $C_{4:0}$ to $C_{12:0}$ accounted for approximately 40% and $C_{16:0}$ was approximately 30% of the total fatty acids synthesized. From seven to 40 days after parturition, there was little change in the composition of fatty acids synthesized in the mammary tissue. It was indicated that between the 18th day prior to parturition and the 20th day after parturition a 30 fold increase was seen in the rate of fatty acid synthesis in mammary gland tissue.

Brumby and Welch (1978) reported research work has indicated that yield of short- and medium-chain length fatty acids, other than $C_{4:0}$, are positively related to carbohydrate and cellulose intake and negatively to fatty acid intake. Yields of $C_{14:0}$, $C_{16:0}$ and C_{18} fatty acids are positively related to cellulose and carbohydrate intake, negatively related to live weight change and curvilinear to fatty acid intake. The authors state that the negative coefficients for the intake of $C_{6:0}$ to $C_{14:0}$ is due to acetate production in the rumen being diminished with increased intake of fatty acid. The regression coefficients for the yield of butyrate, which is formed from both circulating β-hydroxybutyrate and acetate, is probably due to the derivation of β-hydroxybutyrate from the metabolism of long-chain fatty acids as

well as the effect of carbohydrate, cellulose and fatty acid intakes on ruminal acetate production. The curvilinear relationship for the yield of C_{16} and C_{18} fatty acids indicates that the proportion of ingested fatty acid available for transfer to milk diminishes with intake. A decline in these fatty acids could be the result of oxidation of lipid as it replaces carbohydrate and cellulose in the diet, however, evidence is needed to support this explanation.

In summary, the dietary nutrients supplied to the dairy cow influences milk composition by changing the precursor pool available to the mammary gland for milk synthesis. The ruminant digestive system allows milk fat composition to be altered by dietary manipulation. The rumen is a large fermentation vat which results in the growth of microorganisms that utilize feedstuffs as substrates to form other products. Rumen bacteria and protozoa vary in number and type depending upon the amount and type of forages and concentrates supplied to the animal which influences the end products of fermentation. Dietary carbohydrates are converted by rumen microorganisms into VFA that are absorbed into the blood stream and become the primary energy source for the ruminant animal. Volatile fatty acids are also important precursors for milk fat and lactose synthesis. The VFA which have the greatest impact on milk fat synthesis are acetic and propionic acids as well as β-hydroxybutyrate. Lactic acid can also be used as a substrate for fatty acid synthesis.

1.4. Influence of Dietary Lipids on Ruminal Digestion

The effect of dietary lipids on ruminant metabolism has been reviewed (Bauchart, 1993, Jenkins, 1993, Chilliard, 1993, Storry, 1981). The total amount of milk fat depends upon the fatty acids which are derived from intramammary synthesis and from lipoprotein triacylglycerides or unesterified fatty acids of blood plasma. A number of factors can change the contribution of these fatty acids to milk fat. These factors include stage of lactation, quantity of fatty acids, composition and physical form of fat in the diet, the period and frequency of feeding and the roughage (fibre) to concentrate ratio of the diet.

Dietary fat can impact the yield and composition of milk fat. In the rumen, the indirect affects of dietary fat include reduced de novo synthesis of fatty acids by microorganisms leading to a reduced supply of long-chain fatty acids for milk fat synthesis. Another indirect

effect is that rumen fermentation is altered to give a decreased acetate:propionate ratio. Direct effects of dietary fat on metabolism in relation to milk fat synthesis involves hydrolysis and hydrogenation of fatty acids leading to an increased availability of saturated long-chain fatty acids.

Devendra and Lewis (1974) summarized four theories to explain interactions between dietary fat and fibre leading to reduced digestibility in the rumen. The first theory states that reduced fibre digestibility is due to the physical coating of the fibre which prevents microbial attack. Storry (1981) states that fat physically coating the fibre can prevent microbial attack and/or inhibit microbial activity which reduces fibre digestibility in the rumen. The physical coating theory indicated in the review by Jenkins (1993) also indicates that a lipid layer over the feed particles inhibits the digestion of cellulose and results in reduced rumen fermentation. The lipid covering, therefore, causes detrimental effects by inhibiting close contact of microbial cells or their hydrolytic enzymes with the feed particles. In order for feed particles to be digested, the rumen microbes must have physical attachment with the feed particles. Jenkins (1993) also claims that lipids in the rumen can inhibit fermentation by partitioning into the microbial plasma membranes and disrupting their function. Palmquist and Jenkins (1980) claim that fatty acids, particularly polyunsaturates, are inhibitory to microbial growth.

The second theory stated by Devendra and Lewis (1974) arises from studies of prolonged supplementation of 5% maize oil in the diet on crude fibre digestibility. From these studies it was suggested that lipid supplementation modifies the rumen microbial population involved in cellulose digestion. The modification of the rumen microbial population is possibly due to the toxic effects of fat on certain microorganisms. A third theory indicates that there is an inhibition in the activity of rumen microbes due to lipid supplementation. Fatty acids may inhibit or promote growth due to the effect on cell permeability from the absorption of fatty acids on the cell wall. The fourth theory claims that following the addition of lipid supplements to the diet, there is a simultaneous marked depression in fibre digestibility and reduced retention of calcium (Ca) due to excessive excretion of soaps in the feces. It was stated that a reduced availability of Ca and magnesium (Mg) may interfere with microbial activity since these elements have been shown to be essential for microbial growth. Devendra

and Lewis (1974) indicated that Ca is important in relation to crude fibre digestibility in lipid supplemented diets. Depression in crude fibre digestibility can be alleviated by Ca supplementation. Palmquist and Jenkins (1980) interpret this fourth theory as being a reduction in cation availability from the formation of insoluble complexes with long-chain fatty acids. This effect, therefore, could be directly affecting the availability of cations for microbial function or indirectly affecting rumen pH (Palmquist and Jenkins, 1980).

Storry (1981) indicates that the decreased intramammary fatty acid synthesis may be attributed to (1) an altered production of VFA in the rumen, (2) decreasing the amount of acetate and β-hydroxybutyrate to the mammary gland and/or (3) a direct inhibition of mammary ACC activity through increased mammary uptake of long-chain fatty acids from plasma triglycerides. The increased uptake of triglyceride fatty acids in the mammary gland causes an increased yield of long-chain fatty acids in milk. In summary, the balance of the direct and indirect effects (pathways) will influence the yield and composition of milk fat.

1.5. Milk Components

Milk is composed of more than 100 components, the primary ones being protein, lactose, fat, ash, total solids and water (Table 1.1) (Kennelly, 1996a, Bath et al., 1985). The components of bovine milk are derived from two sources: (1) the mammary gland and (2) transfer from blood plasma to milk (Kuzdzal - Savoie et al., 1980). Milk protein, lactose and fat and a variety of enzymes and sugar derivatives are synthesized within the mammary gland from dietary components. Water, minerals, vitamins and products of nitrogen metabolism are transferred directly from the blood plasma to milk (Kuzdzal - Savoie et al., 1980). Milk components can be manipulated, however, the extent of their manipulation varies with the component. Milk composition is influenced by a host of factors which include breed, genetics, stage of lactation, seasonal effects, diet (ie: grain intake, forage:concentrate ratio), feeding frequency and management and protein and energy intake.

The cow derives its supply of amino acids for milk protein production through digestion in the small intestine from dietary proteins which have passed through the rumen

undegraded, rumen synthesized microbial protein and secreted endogenous protein. The composition and amounts of these proteins are determined by the intake and rumen degradability of the dietary protein. In the laboratory, milk protein is measured as non-protein nitrogen (NPN) (urea, amino acids) or as true protein, which encompasses the casein and whey proteins (Kennelly, 1996a). The main constituents of milk protein are casein and whey proteins, blood albumin and immunoglobulins. The casein proteins amount to approximately 2.6% by weight of milk (Kuzdzal - Savoie et al., 1980). Casein proteins can be designated as either α_{s1}-, α_{s2}-, β and κ - casein. The whey proteins are composed of two main constituents, β-lactoglobulin and α-lactalbumin as well as lactoferrins and immuoglobulins. Some typical values for the concentrations of individual milk proteins and their percentage contribution to total milk protein are illustrated in Table 1.2 (Porter, 1980).

Table 1.1. The general composition of bovine milk (%).

Protein	Casein	2.5
	Whey protein	0.5
	Non-protein nitrogen	0.2
		3.2
Fat		3.2
Lactose		5.0
Ash		0.7
Total Solids		12.5
Water		87.5

Changes in milk protein content can be brought about by dietary manipulation such as dietary lipid supplementation (Drackley and Elliott, 1993, Grummer et al., 1993). Lipid supplementation may have a relatively small effect on the protein concentration but it can have a substantial impact on the protein to fat ratio and influence the yields of protein and fat by the dairy cow. The effect on milk protein is attributed to changes in propionic acid production in the rumen (Thomas, 1984).

Lactose is the main osmotically active component of milk and is the predominant

carbohydrate found in milk. Other carbohydrates in milk include monosaccharides and oligosaccharides. Bovine milk contains approximately 4.8 to 5% lactose (Kennelly, 1996a, Brink and Lofgren, 1982). The concentration of lactose in a healthy mammary gland in mid-lactation is relatively constant and the volume of milk secreted is directly proportion to the amount of lactose synthesized (Miller, 1979). Increasing lactose synthesis in the mammary gland results in an increased water secretion and milk volume. Decreased lactose synthesis in the mammary gland, therefore, results in decreased water secretion and milk volume declines.

The most studied component of milk is milk fat. The average composition of milk lipids is given in Table 1.3 (Bitman and Wood, 1990). Jensen et al. (1991) provides a description of the components which comprise milk lipids. In bovine milk, triglycerides, diglycerides and monoglycerides make up approximately 98% of the lipids found in milk fat with phospholipids and sterols comprising 1% and 0.5%, respectively. Fatty acids constitute 90% of the glycerides by weight (Kuzdzal - Savoie et al., 1980). Jensen et al. (1991) and Kuzdzal - Savoie et al. (1980) both state that bovine milk lipid contains 10 major fatty acids which include butyric ($C_{4:0}$), caproic ($C_{6:0}$), capric ($C_{10:0}$), lauric ($C_{12:0}$), myristic ($C_{14:0}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and linolenic ($C_{18:3}$). Four hundred fatty acids have been identified. Triaclyglycerol composition is defined by the amount and kind of fatty acids present (Jensen et al., 1991). The structure of the triaclyglycerol is also important since it influences the action of lipolytic enzymes and, therefore, absorption. Jensen et al. (1991) states that the fatty acid composition (bovine milk triaclyglycerol structure) is greatly affected by dietary changes due to biohydrogenation and production of short-chain fatty acids in the rumen.

Phospholipids make up approximately 1% of the lipid found in bovine milk and originate from the membranes of mammary cells. This group of lipids acts as emulsifiers and milk fat globule membrane stabilizers and are a source of long-chain polyunsaturated fatty acids. Phospholipids also bind cations and may interact with digestive enzymes. Jensen et al. (1991) also indicates that phospholipids are the focus of autoxidation due to their location on the fat globule membrane and polyunsaturated fatty acid content. Cholesterol is

Table 1.2. Typical values for concentrations of individual milk proteins and their percentage contribution to total milk protein.

Protein	Concentration (g/l)	% of total
Caseins	26	79
Whey		
β-lactoglobulin	2.5	7.5
α-lactalbumin	1.5	4.5
Immunoglobulins	1.0	3.0
Blood serum albumin	0.3	1.0
Proteose peptone	1.5	4.5
Total protein	33	

Table 1.3. Average composition of bovine milk lipids.

Lipid class	% of total lipid
Triacylglycerol	95.8
Diacylglycerol	2.25
Phospholipid	1.11
Cholesterol	0.46
Free fatty acids	0.28
Monoacyglycerol	0.08
Cholesterol ester	0.02

the major sterol found in bovine milk fat. Serum cholesterol is derived partly from the diet and partly as a result of synthesis within the body tissues (Porter, 1980). The contribution of cholesterol from these two sources will vary according to the amount of cholesterol and saturated fatty acids found in the diet. Jensen et al. (1990) indicated that cholesterol ranges from 10 to 20 mg/dl with the quantity being related to milk fat content.

Milk fatty acid composition has been extensively studied by such methods as gas liquid chromatography (GLC) or gas chromatography (GC) as butyl or methyl esters. Khorasani

and Kennelly (1996) illustrate the percentage total fatty acid composition of milk lipids for cows in mid-lactation (approximately 150 DIM) as indicated in Table 1.4. From the results obtained by Khorasani and Kennelly (1996) a large portion, approximately 70%, of fatty acids found in milk are saturated. The balance is comprised of monounsaturated and polyunsaturated fatty acids. The majority of saturated fatty acids are $C_{14:0}$ and $C_{16:0}$ acids.

Milk fat has been the primary component for adjusting the price of milk. Traditionally dairy farmers have put considerable time and money into trying to avoid milk fat depression. The fat component of milk is the component that is the most responsive to dietary change and

Table 1.4. The fatty acid composition (% of total fatty acids) of bovine milk.

Fatty Acid	% of total fatty acids	Fatty Acid	% of total fatty acids
$C_{4:0}$	2.48	$C_{16:0}$	33.18
$C_{6:0}$	2.43	$C_{16:1}$	1.81
$C_{8:0}$	1.58	$C_{17:0}$	0.61
$C_{10:0}$	3.42	$C_{18:0}$	7.97
$C_{12:0}$	3.80	$C_{18:1}$	22.00
$C_{14:0}$	12.72	$C_{18:2}$	1.96
$C_{14:1}$	1.56	$C_{18:3}$	0.59
$C_{15:0}$	1.52		

is manipulated over the widest range in comparison to lactose and protein (Rode, 1989). Fatty acid composition and fat percentage can both be changed by nutritional manipulation. Kennelly (1996a) indicates that the quantity and composition of milk fat can be altered relatively independently of changes in milk protein and lactose concentration. Milk fat content and composition, therefore, can be altered immediately if there are sudden changes in the supply of energy substrates to the rumen and mammary gland (Rode, 1989). Sutton (1989) indicates that the concentration of milk fat can be altered by nutritional means over a range of about three percentage units, protein by about one-fifth of this and lactose varies little.

Minor components of milk include vitamins, non-protein nitrogenous compounds and minerals. Examples of some of the vitamins found in milk include vitamin A, D, E, K and C, riboflavin and thiamin. Examples of non-protein nitrogenous compounds found in milk include total NPN, ammonia N and urea. Both macro and trace minerals are found in milk. Examples of macro minerals include calcium (Ca), phosphorous (P), potassium (K) and sodium (Na). Some examples of trace minerals include iron (Fe), manganese (Mn), zinc (Zn) or copper (Cu). Further details on the minor components of milk are discussed by Swaisgood (1982) and Webb et al. (1974).

1.6. Why Alter Milk Composition?

Milk is considered to be one of nature's most perfect and natural foods available to the consumer. This is due to the fact that milk provides a balance of protein, fat, carbohydrates, vitamins and minerals. If milk is able to provide the consumer and the animal's offspring with a balanced source of essential nutrients, the question remains: "Why alter milk composition?". Hettinga (1989) indicates a number of reasons for altering milk composition such as preservation, economics, processing, marketing, lifestyle and nutrition. Preservation allows a number of dairy products to be produced and transported great distances from the farm gate. Economics plays a role in the pricing system of milk. The pricing system in Canada and the U.S. is based upon its components, mainly the fat component, however, producers are being paid for the protein component. Another economic reason for altering milk composition is the increased number of products which can be manufactured from milk and sold to the consumer. This can provide the manufacturer with greater economic returns by having a variety of products available in the market place.

Lifestyle influences the question of why milk composition should or should not be altered. In a current review by Kaylegian et al. (1993), the authors indicate that milk fat possesses a desirable flavour and is perceived by the consumer as a natural and high quality product. Consumers are, however, concerned about their intake of fat, cholesterol, calories and the nutritional value of food. The medical profession has linked dietary fat to cardiovascular disease. Health concerns have resulted in a decline in milk fat consumption

due to its high level of saturated fat and hypercholestrolemic qualities, however, milk fat is not high in cholesterol (Jensen et al., 1991). For example, consumers have shifted from whole milk to 2%, 1% or skim milk. Hettinga (1989) states that a survey found that 53% of the respondents consumed low fat milk for diet or caloric reasons, 26% for health or fat reasons, 15% for taste and 7% due to price. Kennelly (1996a) states that in 1993, 75% of total milk fat sales in Canada were in the form of low-fat milk, whereas, in the U.K low-fat sales increased from approximately 2% of all milk sales in 1983 to 45% of milk sales in 1991. The decline in milk fat consumption, however, has been slow to change the milk pricing system to encourage production of lower milk fat. The decline in milk fat consumption has resulted in the focus being primarily on special incentive and promotion programs to encourage the use of milk fat (Kennelly, 1996a).

As previously noted, the composition of typical milk fat is 70% saturated, 25% monounsaturated and 5% polyunsaturated fatty acids (Grummer, 1991). Milk fat has, therefore, been identified as being a hypercholesterolemic fat since it contains cholesterol and has primarily saturated fatty acids. Of the saturated fatty acids, approximately 10-15% are short- and medium-chain fatty acids composed of 12 or less carbons (Khorasani and Kennelly, 1996, Harrison et al., 1995, Kim et al., 1993). Hypercholestrolemic effects of saturated fats in human diets is largely due to the effect of $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$ (Kennelly, 1996b, Ashes et al., 1995, Ney, 1991). Grundy and Denke (1990) state that data on $C_{12:0}$ is contradictory and this acid may be hypocholestrolemic. Data reported by Hayes et al. (1991), however, reports $C_{12:0}$ and $C_{14:0}$ being hypercholestrolemic but $C_{16:0}$ is less cholestrolemic than $C_{12:0}$ plus $C_{14:0}$. Grundy and Denke (1990) indicate that $C_{16:0}$ has been shown to produce elevated levels of total plasma and LDL cholesterol. The American Heart Association (1990) states there is a concern about the relatively high proportion of specific fatty acids, such as $C_{14:0}$ and $C_{16:0}$, in milk and meat products due to their role in elevating LDL and the association with cardiovascular disease. Consumption of $C_{16:0}$ and possibly $C_{12:0}$ and $C_{14:0}$ in amounts greater than 10% of total calories can increase plasma cholesterol levels but this effect can be reduced by the consumption of $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$ in dairy products and other foods (Grundy and Denke, 1990). Saturated fatty acids with less than 12 carbons or saturated or

monounsaturated fatty acids with 18 carbons do not raise blood cholesterol relative to polyunsaturated fatty acids (Ney, 1991, Grundy and Denke, 1990).

Kennelly (1996b) reports that $C_{18:0}$ and $C_{18:1}$ are two fatty acids which were previously targeted as being undesirable but are now considered to be neutral or positive from a human health perspective. Jensen et al. (1991) comments that $C_{18:0}$ has been shown to be as effective as $C_{18:1}$ in reducing plasma cholesterol. Dietary fatty acids do not all have the same effect on plasma cholesterol levels in comparison to polyunsaturated fatty acids (Ney, 1991). Researchers have stated that each fatty acid influences a different step in the development of cardiovascular disease and other chronic diseases, therefore, the interaction among fatty acids has a profound impact on these disorders (O'Donnell, 1993). Dietary fatty acids contain a mixture of fatty acids and certain fatty acids may be predominate in a particular fat, therefore, this adds to the already complex interactions of dietary fatty acids and cholesterol metabolism. The medical profession, therefore, emphasizes to consumers to reduce their consumption of saturated fat, especially products containing high proportions of $C_{16:0}$, $C_{14:0}$ or $C_{12:0}$ (O'Donnell, 1993).

In summary, consumer concerns have resulted in a significant impact on the food industry. Consumers are moving towards diets containing lower fat and cholesterol resulting in a shift from animal and tropical fats toward more unsaturated vegetable fats and oils. The impact of what the consumer desires has resulted in large number of low fat and low cholesterol products in the marketplace. Health concerns have also influenced and encouraged manufacturers to reformulate products to replace saturated tropical oils with more unsaturated fats (Kaylegian et al., 1993). Milk fat has been given a negative health image and in order to correct this issue the dairy industry needs to educate the consumer in how to incorporate milk fat into a healthy diet. It has been indicated through nutritional research studies that short- and medium-chain fatty acids are metabolized differently than the long-chain fatty acids and not all saturated fatty acids are detrimental to human health as once thought (Kaylegian et al., 1993). O'Donnell (1993) indicates that understanding both milk fat chemistry and the body's physiological chemistry will allow manufacturers and product developers to modify milk fat to meet the changing functional and nutritional targets

demanded by the consumer. Ashes et al. (1995) states that the consumer demand for milk products with specific qualities has influenced and will continue to influence the overall feeding strategies for ruminants.

1.7. Protected Fat Sources and Their Background

Using dietary fat in the diet of lactating dairy cows is not a new area of research. A great number of studies have been conducted to investigate the effect of individual or combinations of fat sources on the fatty acid composition of milk. There are too many studies in the literature to discuss them individually or as a group and this is beyond the scope of this literature review. In general, various studies using either animal fat sources (ie: tallow) or oilseed sources (ie: soybean, cottonseed, sunflower, safflower, flaxseed and canola) as unprotected fat sources have produced similar effects in terms of milk fatty acid composition. The addition of these fat sources has resulted in reducing short- and medium-chain fatty acids and subsequently increasing long-chain fatty acids in milk fat. The main emphasis of dietary manipulation of milk fat, therefore, is to decrease the concentration of $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$ while increasing the long-chain fatty acids, such as $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$. Dietary manipulation, therefore, results in increased long-chain fatty acids at the expense of $C_{14:0}$ and $C_{16:0}$ and this is considered desirable from a human health perspective. As long as the consumer is being told to reduce the amount of fat and calories in his/her diet, this message is being interpreted as reducing the amount of fat in the foods consumed. O'Donnell (1993) comments that as society addresses dietary issues, the dairy industry must be prepared to modify its products to meet consumer demands. Also, public health agencies will continue to recommend new approaches to maintaining desirable body composition which will include dietary manipulations based on new nutritional information. An investment in continued research will prepare the dairy industry for future consumption of dairy products based on consumer demand and their effects on human health. This will allow the dairy industry to be proactive and remain competitive.

Fat sources can also be protected against rumen biohydrogenation in order to alter milk fat fatty acid composition. In order to achieve this goal, the unsaturated fatty acids must

be fed in a form which will resist rumen biohydrogenation. By feeding protected fat to dairy cattle, the concentration of short- and medium-chain fatty acids in milk can be reduced while increasing the long-chain fatty acids. The fatty acid composition, and in particular, the unsaturated fatty acid profile, of the protected fat is important due to the fact that the fatty acid composition of milk fat changes in the direction of the protected lipid (Fogerty and Johnson, 1980). This point must be remembered since each oilseed or animal fat has its own distinctive fatty acid composition. There are a number of reasons for including protected fats in the diet of dairy cattle such as: (1) to increase the energy density of the diet, especially during early-lactation when dairy cows are in negative energy balance, (2) to improve efficiency of fat utilization for lactation (the C_{16} to C_{22} fatty acids are directly transferred to the milk fat), (3) to improve persistency in milk yield and reduce ketosis and (4) to manipulate the fatty acid composition of milk and milk products (Scott and Ashes, 1993). Scott and Ashes (1993) also indicate that there are a number of factors which need to be considered and evaluated when selecting the type of fat to be protected: (1) the effects of "free fat" on rumen microorganisms - the higher the degree of unsaturation in the fatty acids, the more deleterious is the effect on rumen fermentation, (2) efficiency of absorption from the small intestine, (3) utilization of absorbed fatty acids and the consequent metabolic effects, (4) effects on the fatty acid profiles of tissue/milk lipids and the formation of lipid derived 'second messengers' in signal transduction pathways and (5) nutritional characteristics, quality and organoleptic properties of the meat and milk produced.

A number of factors will affect the transfer efficiencies of fatty acids from the diet to the milk. These factors can apply to both unprotected and protected dietary fat sources and include: (1) stage of lactation, (2) basal diet, (3) level of lipid intake, (4) hydrogenation of unsaturated fatty acids by rumen microorganisms, (5) hydrolysis of dietary esterified fatty acids, (6) postruminal lipid digestion and (7) intestinal and mammary desaturase activity. Hydrogenation of dietary unsaturated fatty acids by ruminal microorganisms negatively affects the fatty acid composition of milk fat. In order to alleviate the inhibitory effects of the hydrogenation of dietary unsaturated fatty acids by microorganisms and the effects of fat on ruminal digestion, one can utilize encapsulation, feeding of full-fat oilseeds or protecting the

fat from microbial attack in the rumen using chemical or heat treatment of the oilseed (Kennelly and Khorasani, 1992).

If nutritionists are so interested in protected lipids a question to ask is "What is a protected lipid?". Storry et al (1974) defines a formaldehyde protected lipid as: "Oil or fat droplets are encapsulated in a layer of aldehyde treated protein, which, because, of cross-linking between free amino groups, is relatively insoluble and inert to bacterial breakdown in the rumen. Under the acidic conditions of the abomasum, however, the encapsulated oil droplets are released and made available for enzymatic digestion and absorption in the small intestine". Ashes et al. (1979) states that the essential features of a biologically protected lipid source are: (1) the lipid droplets must be firmly embedded in a continuous matrix or protein, (2) the formaldehyde must react with the protein in order to prevent protein solubility, (3) there must be sufficient reversibility of the formaldehyde reaction so that the protein is solubilized after passage through the rumen and (4) absorption of the lipid is from the small intestine. Overprotection of the lipid source, however, can result in some of the lipid passing undigested through the digestive tract. Underprotecting the lipid source can result in less polyunsaturated fats being able to withstand bacterial attack in the rumen.

1.7.1. Chemical Treatment

The process of protected lipid sources originated in Australia from the combination of two unrelated techniques. These two techniques involved the protection of protein and the production of spray-dried butter powder. One technique involved using formaldehyde to protect casein from ruminal breakdown. The other technique involved spray-dried butter powder which had used butter emulsified with sodium caseinate resulting in the minute droplets of milk fat to be coated with a thin film of casein (Fogerty and Johnson, 1980, Harrap, 1973). When protected lipid supplements are used, the protein as well as the fat is protected, and the formaldehyde treatment prevents the destruction of some of the amino acids in the rumen (Dunkley et al., 1977).

Scott et al. (1971) provides a description of a procedure used to create protected lipids. A recent paper by Scott and Ashes (1993) indicates that the process of producing fats

encapsulated in a matrix of aldehyde-treated proteins has been re-engineered from its original procedure. The process now involves computer aided control systems to improve the manufacturing procedures and the quality of the fat supplements. The current system involves mixing the oilseed together with an antioxidant which is homogenized with water. Alkali is introduced to the mixture to solubilize the protein. The slurry contains approximately 45% solids and is then treated with formaldehyde. The slurry is dried prior to feeding. This procedure was utilized to produce the protected formaldehyde treated canola seed used for the current study (Ashes, 1996).

Once the protected feed supplement is created in a form of a dried powder it can then be added to the diet. The process of digestion is similar to that of unprotected lipid sources with one major difference - the protected lipid is resistant to the attack of microbial organisms in the rumen. When the protected supplement is ingested by the ruminant animal, the formaldehyde-protein coating of the oil droplet resists microbial attack in the rumen. When the protected supplement enters the abomasum, the acidic conditions (pH 2-3) cause the formaldehyde-protein bonds to split which releases the protein and encapsulated lipid (Jensen et al., 1991, Fogerty and Johnson, 1980). Once the bonds are broken, the protein and lipid can be digested and absorbed into the tissues. Protection of this type results in biomanipulation in which digestive processes of supplements fed to ruminant animals can be modified to resemble those of a monogastric animal in terms of fatty acid composition of the tissue or milk (Bauchart, 1993, Fogerty and Johnson, 1980).

The most effective way to consistently and substantially increase the proportions of C_{18} mono and/or polyunsaturated fatty acids and reduce the concentration of $C_{12:0}$ - $C_{16:0}$ is to feed oilseeds which have the constituent C_{18} unsaturated fatty acids highly protected from ruminal biohydrogenation by encapsulation in a matrix of formaldehyde treated protein (Ashes et al., 1995, 1992, McDonald and Scott, 1977). Feeding of fats and proteins effectively protected from ruminal digestion (1) allows an increase in energy density (2) eliminates or reduces deleterious effects on microbial function, (3) enhances the supply of protein and energy for absorption and utilization and (4) manipulates the fatty acid composition of milk and milk products (Ashes et al., 1995). Ashes et al. (1995) also states that the technology is

available to significantly alter the overall composition of milk, however, the challenge is to design fat supplements and strategies to change the structure of milk triaclyglycerols to produce a wide range of fats with different combinations of saturated and unsaturated fatty acids. This will result in producing milk products with a wide range of physical properties (ie: soft butter) and increase the scope and availability of milk fat in the marketplace.

A concern with using formaldehyde as a protection method for oilseeds or animal fat sources is the possibility that formaldehyde is transferred to the milk. In an experiment by Wrenn et al. (1976) a formaldehyde protected sunflower-soybean supplement was fed to dairy cows. Results of this study determined that the amount of formaldehyde in milk when the protected supplement was fed ranged between 0.1 to 0.3 μg/ml and no more than 0.023% was recovered from milk (amount detected minus background amounts in milk when no formaldehyde was fed). Wrenn et al. (1977) fed a formaldehyde protected fat supplement for a period of two years and their results indicated that the amount of formaldehyde present in milk was 0.22 ppm. Both studies indicate that the amount of formaldehyde transferred to milk was relatively small. Current research by Atwal and Mahadevan (1994) found that feeding formaldehyde treated soybean meal did not affect the level of formaldehyde naturally detected in milk. Atwal and Mahadevan (1994) indicate that research states formaldehyde levels range from 0.017 mg/kg to 0.2 mg/kg in cow's milk. Wrenn et al. (1976) stated that most of the formaldehyde fed in the experimental diets was eliminated as gaseous waste. Kaminski et al. (1993) states there are low (up to 0.027 mg/kg) but persistent levels of formaldehyde in milk of cows fed typical diets without added formaldehyde. Other studies which have investigated the effect of formaldehyde protected supplements have not reported values for the amount of formaldehyde transferred to milk or indicated any adverse health problems due to feeding formaldehyde protected supplements.

1.7.2. Heat Treatment

Another way in which fat supplements can be protected from ruminal biohydrogenation is by using heat treatment. Heat treatment can involve various temperatures with various moisture levels and/or combinations of heat with or without moisture. Jet-

sploding involves heating the product with recirculated hot air at a temperature of approximately 315°C and as the product leaves the unit it passes through a roller which causes the product to rupture (Marty and Chavez, 1993). Jet-Sploding which involves high temperature for a short period of time and utilizes only the moisture within the seed (Deacon et al., 1988). Marty and Chavez (1993) subjected soybean to this process and observed average exit temperatures of 149-163°C and a high internal vapour pressure. Emanuelson et al. (1991) states that treating whole seed with heat and/or steam can change the physical structure of the fat conglomerates. This could cause an advantageous increase in the amount of rumen undegradable protein and possibly decrease the negative effects of fat on rumen fibre or organic matter digestibility. Heat treatment decreases the solubility of proteins and creates cross linkages both within and among peptide chains and to carbohydrates (Deacon et al., 1988). High temperatures and extended heating times, however, can result in the protein being less digestible due to the Maillard reaction between sugar aldehyde groups and free amino acids, thus, lowering the digestibility of the amino acids (Deacon et al., 1988).

The term Maillard reaction or nonezymatic browning is used to describe the complex series of chemical reactions between the carbonyl and amino component derived from biological systems (Mottram, 1994, Ledl, 1990). Fox et al. (1983) describes the steps involoved in the Maillard reaction. The initial step of the Maillard reaction is the condensation of an animo component and a reducing sugar resulting in the formation of N-Glycoside and the splitting off of water. Several amino acids undergo the Maillard reaction, in particular lysine and methionine (Fox and Cameron, 1982). The glycoside undergoes an Amadori arrangement in which a keto dervative is formed. The first step of the Maillard reaction is a first order reaction and leads to a nutrient loss in food since the amino acid and protein which react with the sugar are no long available for metabolism. In foodstuffs, the Maillard reaction is responsible for changes in flavour, color or nutritive value and the formation of stabilizing or mutagenic compounds (Ledl, 1990). The colorless product formed during the first phase of the reaction reacts further with amino components and polymerizes into the brown pigment melanoiden. Devlopment of the brown pigment in the later phase of the reaction is influenced extensively by reaction conditions such as pH, time and temperature

of the thermal process and available water (Fox et al., 1983). For example, excess heating generally leads to the destruction of amino acids and vitamins, browing and the development of cooked flavour (Kwok and Niranjan, 1995).

Belitz and Grosch (1987) indicate that heating protein in a dry state at neutral pH caused the Maillard reaction to form isopeptice bonds between lysine and residues and the β- and γ-carboxamide group of asparagine and glutamine residues. The isopeptide bonds are cleaved during acid hydrolysis of protein, therefore, resulting in degistible amino acids. Additional heat results in more extensive protein denaturation. Van Soest (1982) and Waller and Feather (1983) indicate pH and water seem to be the most important factors, in association with heat, in determining reaction rate. The Maillard reaction occurs at particularly high temperatures and at pH values of seven and above, a certain amount of moisture is required (Fox and Cameron, 1982). Research of Lee et al. (1984) indicates Maillard reaction polymers increase with increased pH and breakpoints in polymer formation ocurred at pH 6 and 5 at temperature of 100 and 110°C, respectively. Fox et al. (1983) also found that highly acidic medium (pH 3), the Maillard reaction had a much longer lag period than at pH 5 and 7 and the effect of temperature (85-95°C) had no effect on the reaction.

A second method for heat treating is extrusion. Extrusion cooking utilizes friction as the source of heat accompanied by pressure and attrition (Marty and Cahvez, 1993). Heat and pressure are generated by passing the material through a barrel by a means of a screw with increasing resistance and finally discharging the product from the exturder. Examples of exturders include single or twin-screw. Single and twin screw extruders perform the function of introducing significant quantities of energy into the extrudate (Levine, 1992). The energy addition results in the physical and chemical changes to the extrudate such as expansion (puffing), starch gelatinization and protein denaturation (Levine, 1992).

Micronization involves exposure to infrared (radiant) energy which is electromagnetic radiation with a wider frequency range than microwaves and is emitted by hot objects (Fellows, 1988). Infrared energy gives up its energy to heat the material when it is absorbed which is in contrast to the more penetrating microwaves that induce molecular friction. Marty and Chavez (1993) exposed full fat soybean to infrared energy for 90 sec during which

temperatures of 110-115°C were obtained.

Another method of heat treatment involves roasting the material. Roasting consists of passing the product through a gas fired chamber. In the study of Marty and Chavez (1993) full fat soybean had a seed temperature of 110-130°C upon exiting the chamber after approximately 2-5 min after entering the roaster.

1.7.3. Other Methods of Protection of Lipid Supplements

Other methods of protecting lipid supplements include using Ca soaps of palm oil fatty acids or prilling of the fat source. Calcium soaps of palm oil fatty acids (ie: Megalac®) are stable in the rumen environment and provide effective protection against rumen biohydrogenation. Megalac® is a dry granular material that mixes with common feed ingredients. This bypass fat is in the form of non-melting granules. Results of a study by Wu et al. (1991) indicated that high levels of Ca soaps in the diet (6% of DM) can lower intestinal digestibility of fatty acids. Prilling of a fat source involves the creation of a pellet. Grummer (1988) stated that prilled fat as well as Ca salts of palm oil fatty acids are inert in the rumen when supplemented at 3.5% or less of the total ration DM. Alifet is another example of a rumen bypass fat supplement which is produced on a crystallization tower. This product is produced from higher quality animal fat combined with starch to create free flowing crystals which is added to dairy feeds throughout lactation.

Due to the relatively high cost of using a protected lipid source whole oilseeds can be utilized. The whole oilseed is ground or crushed and can provide a possible inexpensive alternative to utilizing a protected lipid in the ration of the dairy cow.

1.7.4. Which Protection Method to Use in Order to Alter Milk Fatty Acid Composition?

Changes in milk fatty acid composition have been observed with the use of protected fats and various research studies have confirmed that protected supplements decrease the short-chain fatty acids and increase the polyunsaturated fatty acids found in milk. Experiments have indicated that protected fat sources are able to escape rumen degradation

and biohydrogenation, be degraded in the abomasum and the fatty acids transferred to the mammary gland (Khorasani and Kennelly, 1996, Harrison et al., 1995, Ashes et al., 1992) This results in increased unsaturated fatty acids in the milk and a decrease in the concentration of saturated fatty acids.

Selection of a protected lipid supplement will depend upon the protection method of interest, availability of the protected lipid source and cost. Two methods which were utilized in this current study involved the use of formaldehyde and heat treated canola seed. Formaldehyde and heat treatment of canola seed were both selected on the basis that they are used to increase the extent of rumen escape of unsaturated fatty acids. For the formaldehyde protected supplements, improved technology and using whole oilseeds as a substitute for casein and a reduction in production costs has resulted in permitting the production process to be more commercially viable. Production plants have been established in Australia and New Zealand. Presently in Alberta, the production of formaldehyde protected lipid is not done. Using formaldehyde protected oilseeds has been very successful in altering milk fat composition as indicated by the data in the literature (Ashes et al., 1992, Cadden and Kennelly, 1984). Heat treatment of canola seed was chosen as an alternative method to study in comparison to the formaldehyde treated canola seed. Heat treatment has also been successful in altering the fatty acid composition of milk as indicated by the data in the literature (Khorasani and Kennelly, 1996, Khorasani et al., 1991). Also, heat treated canola seed was selected as the technology is commercially available in Alberta.

Utilization of a protected lipid supplement can be influenced by a number of factors: (1) protection method, (2) level of inclusion, (3) diet variation, (4) animal variation, (5) digestibility and ruminal degradation and (6) fat source. These factors need to be considered when studying the data presented by various authors. In the studies which compared unprotected fat sources to protected fat sources, it was illustrated that the protected supplements can decrease short- and medium-chain fatty acids to a greater extent and increase the concentrations of long-chain fatty acids to a greater extent, especially the polyunsaturated fatty acids in milk fat.

1.8. Implications of Feeding Protected Lipid Supplements to Dairy Cows

From the research provided for both unprotected and protected lipid sources, the protected lipids have the advantage of increasing the polyunsaturation of milk greater than unprotected sources. Both forms of lipid supplements result in a decrease in the short- and medium-chain fatty acids while increasing the levels of long-chain fatty acids, therefore, there is a decline in the saturated fatty acids accompanied by an increase in the unsaturated fatty acids. The extent to which dietary lipid sources are protected from microbial degradation in the rumen is an important factor in influencing the amount and extent of change in the fatty acid composition of milk and milk fat yield. Another important factor to consider with the use of protected fat sources is the inclusion level in the dairy cow's diet. If fat is protected from ruminal biohydrogenation then the inclusion level could be greater than if an unprotected fat source was fed. Determination of the optimum inclusion level needs to be considered and determined. The need to change fatty acid composition and fat yield of cow's milk is due to increasing consumer demand for products low in saturated fats, medical professionals linking saturated fatty acids to cardiovascular disease and the consumer trend for substitution of whole milk for 2%, 1% or skim milk. If the nutritionist can devise a feed source which can reduce the amount of saturated fatty acids in milk, the nutritionist has accomplished a major task and can provide the consumer with what they are demanding.

If a suitable protected fat source can be produced and provided to the dairy cow a designer milk may be achievable in the future. Not only are consumers, medical professionals and nutritionists going to benefit from the production of a protected fat source but the producer (both the dairy farmer and cereal farmers), feed mills, chemical companies, food processing plants and manufacturers of the equipment to apply the protection (ie: application of formaldehyde, heat or casein) will also benefit. It is possible that producers would be paid a higher price for milk produced from protected lipid supplements.

Feeding lipid supplements has resulted in an increased concentration of polyunsaturated fatty acids, making the milk more susceptible to autoxidation. In order to overcome the problems of autoxidation the dairy cow diet can be supplemented with α-tocopherol. Charmely and Nicholson (1993) and Charmely et al. (1993) both indicated that

increasing the level of α-tocopherol in milk by intramuscular injection or dietary supplementation, respectively, has been successful in protecting milk from oxidation. Stegeman et al. (1992) and Cadden and Kennelly (1984) found that the addition of supplemental fat resulted in the production of softer butters. Stegeman et al. (1992) stated that rancid off-flavours were not detected in any of the butters after 0 or 3 months of storage. Peroxide values for butter indicated that at 0 or 3 months of storage resulted in no oxidation. McDonald and Scott (1977) indicate polyunsaturated butter is more susceptible to oxidation when exposed to light. Other approaches to reduce oxidation have included direct addition of antioxidants to milk and modifying the processing system involved in the production of butter, cheese and other dairy products. McDonald and Scott (1977) stated that increasing the level of polyunsaturated fats in milk results in softer butter, however, its color was lighter and at temperatures greater than 10°C it tended to break down and oil rapidly. Stegeman et al. (1992) indicated that feeding unsaturated dietary fat was equal or superior in quality to the control milk. Flavour and processing characteristics of butter were also considered to be acceptable.

A challenge for the future of commercial production of milk which contains elevated levels of monounsaturated and polyunsaturated fatty acids is a separate collection system. At the current time, the milk processing sector has yet to investigate the potential market for the production of designer milk and its subsequent products. With pressure from the medical profession to decrease the amount of saturated fat in the diet and the consumer trend to consume low fat products, milk with elevated levels of polyunsaturated fatty acids may greatly influence the market of these products. Interest in this area may also prove to become commercially viable.

In the article by Ashes et al. (1995), the authors indicate that despite having the technology to create protected lipid supplements, a number of factors need further research in order to optimize the use of protected nutrients for improved animal performance and product quality. The following areas require attention in terms of continued research: (1) designing fat supplements in terms of fatty acid composition and the minimum degrees of protection that can be used without affecting cellulose digestion, microbial protein synthesis

or absorption of long-chain fatty acids, (2) examining the interactions between protected nutrients and other dietary ingredients including the fibre:concentrate ratios, fibre structure in respect to degradation rates and residual free fat, proportions of divalent cations and the need for additional fat soluble vitamins, (3) investigating the requirements for other protected nutrients during different stages of lactation and growth as well as compensatory changes and fattening and how these requirements are influenced by genotype and environmental interactions, (4) defining the role and benefit of protected lipid supplements in the diets of ruminants in hot and cold environments and (5) using protected supplements in combination with other dietary constituents to manipulate the amount of rumen degradable and nondegradable protein and energy sources with the aim of producing desired carcass traits such as higher lean:fat content, greater intramuscular deposition, softer butter or fat-modified beef with high proportions of C_{18} mono and polyunsaturated fatty acids.

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CHAPTER 2

The Effect of Formaldehyde and Heat Treatment on Extent of Rumen Escape and Degradation of Canola Seed Protein and Lipid[1].

2.1. Introduction

A quick, effective and widely accepted method to determine the rate and extent of ruminal digestion of a feedstuff is the nylon bag technique. The nylon bag technique involves the incubation of a feedstuff(s) in a synthetic nylon bag which is suspended in the rumen for varying lengths of time. The pore size (50 μm) of the bag allows bacteria to enter and digest the feed. The nylon bag technique permits a large number of samples to be evaluated and is suitable for the initial evaluation of a feedstuff(s). The nylon bag technique essentially measures the rate of disappearance from the bag rather than the actual degradation (Kennelly and Ha, 1983, 1982). The extent of degradation determines both the degradable portion available for rumen microorganisms and the undegradable protein which may be available for the host animal's enzymatic digestion in the intestine. The extent of degradation as well as the rate of degradation are extremely important when nylon bags are used to study the disappearance and degradability of a feedstuff(s).

To determine disappearance of a feedstuff from the nylon bag, Ørskov and McDonald (1979) developed the following equation $p = a + b\ (1-e^{-ct})$ which provides an empirical fit to incubation data. This equation can be derived theoretically from the following assumptions: (1) that there exists one protein fraction (a, percent total protein) which disappears very rapidly within the period before the earliest removal of a bag from the rumen and (2) that a second protein fraction (b, percent total protein) disappears at a constant fractional rate (c) per unit of time. The letter c, therefore, indicates the degradability or disappearance of the component being studied such as the percentage of protein disappearance at time t. Ørskov and McDonald (1979) indicate that the degradability is measured under conditions preventing

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[1]The data was presented in abstract form at the Canadian Society of Animal Science Annual Meeting, Lethbridge, Alberta, Canada, July 7-10, 1996.

the passage of any of the particles from the rumen and is, therefore, an over-estimation of the extent of degradation at any given time under normal conditions when some particles have already passed to the abomasum. The equation is constrained so that the estimates of a and b do not exceed 100%. When calculating degradability, the percentage of disappearance is adjusted to allow for rate of passage of particles from the rumen, thus, effective degradability of nutrients (ie: DM, CP) is calculated by the equation of Ørskov and McDonald (1979) as:

$$EDDM, EDCP = a + ((b \times c)/ (c+k))$$

where k is the estimated rate of outflow from the rumen. Ørskov and McDonald (1979) have assumed that the rapidly disappearing fraction is completely degraded in the rumen. The authors believe this is a reasonable approximation since most of the early loss must consist of water-soluble proteins which are known to be degraded very rapidly.

Feedstuffs, in particular the protein portion of the feedstuff, can be protected from microbial degradation such that the feedstuff can be digested and absorbed postruminally. Methods which are used to protect the protein portion of the feedstuff include heat or chemical treatment. Heat treatment can utilize different temperatures and/or moisture levels. An example of heat treatment is a method known as Jet-Sploding® which uses high temperature (315°C) for a short period of time and utilizes the moisture within the seed (Deacon et al., 1988). The process of heat treatment decreases the solubility of the proteins by creating cross-linkages both within and among peptide chains to carbohydrates, therefore, lowering susceptibility to ruminal degradation (Deacon et al., 1988). An example of chemical treatment of a feedstuff involves the use of formaldehyde as the protecting agent. Formaldehyde reacts with the terminal amino groups of protein and epsilon amino groups of lysine, resulting in methylene bridge formation and rendering the protein more resistant to microbial degradation (Weakley et al., 1983). Treating a feedstuff with formaldehyde results in the formaldehyde forming chemical linkages with the amino acid residues which decrease the solubility of the protein at ruminal pH. Treating canola seed with formaldehyde or heat reduces the extent of degradation in the rumen, thus, both the lipid and protein are available for absorption in the small intestine.

The hypothesis for this current study is that feeding a protected lipid supplement can

be utilized to decrease the concentration of short- and medium-chain fatty acids in milk fat and increase the long-chain unsaturated fatty acids and polyunsaturated acids. The purpose of the nylon bag study was to determine the efficacy of heat and formaldehyde treatment as a means of reducing the rate and extent of digestion of canola seed in the rumen. Lower ruminal disappearance of nutrients is representative of higher rumen bypass or protection from bacterial digestion, therefore, the nylon bag technique can be utilized to compare the degree of protection for heat or formaldehyde treatment of canola seed. The objective of this study was to examine the effect of formaldehyde and heat treated canola seed on ruminal disappearance of dry matter, crude protein and lipid as determined by the *in situ* nylon bag technique (de Boer et al., 1987).

2.2. Materials and Methods

2.2.1. Canola Seed Treatments

The formaldehyde treated canola seed used in this study was acquired from Dr. John Ashes of the Commonwealth Scientific Industrial Research Organization (CSIRO), Blacktown, New South Wales, Australia. The procedure for formaldehyde treatment of oilseeds involves computer aided control systems which mix the oilseed (dehulled) together with an antioxidant. The mixture is homogenized with water and alkali is used to solubilize the protein as outlined by procedures of Scott and Ashes (1993) and Ashes et al. (1992). The formaldehyde treated canola seed was comprised of 70% canola seed, 30% full fat soybean and an antioxidant, ethoxyquin, at 600 ppm. The untreated canola seed and the heat treated canola seed were both acquired from Mary Lou Swift of ProFORM Feeds Inc., Chilliwack, British Columbia, Canada. The heat treated canola seed was coarsely ground and heated at 120°C for 15 min. No moisture was added during the heating process.

2.2.2. Animals and Treatments

Three ruminally cannulated (#1C-rumen cannula with rolled inner flange, 10 cm centre diameter, Bar Diamond Inc., Parma, ID) early-lactation Holstein cows (82 ± 20 DIM) were used. The care of the dairy cows was in accordance with the Animal Use for Research or

Teaching Protocol approved by the Animal Use Committee at the University of Alberta, Edmonton, Alberta, Canada. The diet consisted of 17.5% alfalfa silage, 17.5% pea triticale silage, 10% alfalfa hay and 55% concentrate supplied by Champion Feeds, Barrhead, Alberta, Canada. The canola seed treatments used for the *in situ* nylon bag experiment were untreated canola seed, heat treated canola seed and formaldehyde treated canola seed. Untreated canola seed was used as a control. The three canola seed treatments were ground to pass a 2 mm screen (Fritz® Mill Model D Serial #8972 Comminutor by The Fritzpatrick Company) and 5 g of ground sample was put into the large nylon bag (ANKOM, Fairport, New York, USA). The large nylon bags (n= 54) (5 cm x 10 cm, pore size: 50 ± 15 μm) were sealed with a plastic tie and incubated in the rumen in a polyester mesh lingerie bag (18 cm x 30 cm, mesh size: 3 mm). The incubation times were 0.1, 2, 4, 8, 16 and 24 h. The nylon bags were inserted into the rumen in reverse order of time and were removed after the last incubation time. The actual composition of the canola seed treatments is indicated in Table 2.1.

2.2.3. Analytical Procedures

After the bags were removed from the rumen, they were hand squeezed to remove excess rumen fluid, rinsed with water and frozen. The nylon bag samples were then thawed and washed in a mesh laundry bag in a conventional washing machine on a regular wash cycle (cycle time approximately 8 min). The dirty water was siphoned off and clean water was added and the cycle repeated until the water was colorless. The nylon bag samples were not allowed to spin or spin-rinse during the washing cycle in order to prevent breakage. The water was hand squeezed out of the bags and the bags were placed into pyrex dishes and dried at 60°C in a forced air oven until complete dryness. Samples were removed from the oven, allowed to cool for 24 h and weighed. The contents of the nylon bag was removed and stored in plastic nalgene containers until further analysis.

Dry matter (DM) was determined by drying at 110°C to a constant weight. Crude protein (CP) content was determined using the Kjeldahl procedure (Bradstreet, 1965). Lipid content was determined using the Modified Folch Lipid Extraction Method (Bitman et al., 1983) with the following modifications indicated in Appendix 2.1. The fat percentage was

Table 2.1. Composition of untreated canola seed, heat treated canola seed and formaldehyde treated canola seed used for *in situ* nylon bag technique.

	Canola Seed Treatments		
	Untreated	Heat treated	Formaldehyde treated
Composition, %			
Dry Matter	91.7	89.2	93.3
Crude Protein	22.3	23.1	26.3
ADIN			
% CP	1.8	1.4	1.2
% Total CP	8.1	6.1	4.6
Fibre			
NDF	38.0	33.6	20.4
ADF	33.7	26.0	15.2
Lignin	15.9	13.6	5.8
Hemicellulose	4.8	7.1	5.2
Cellulose	17.9	12.4	9.4
Fat	48.9	44.9	33.0
Fatty Acid, %			
$C_{16:0}$	4.1	4.2	5.3
$C_{18:1}$	58.7	57.8	61.8
$C_{18:2}$	20.9	21.4	18.2
$C_{18:3}$	10.9	11.0	8.7

corrected for DM. Acid detergent insouble nitrogen (ADIN) involved weighing 1 g of sample and boiling in ADF solution for 1 h, filtering and determing N content by the Kjeldahl procedure (AOAC, 1984). Fibre content was determined according to Van Soest et al. (1991). The percentage of disappearance of DM, CP and fat at each incubation time was calculated from the proportion remaining after incubation in the rumen. The disappearance rate of DM (DMDIS), CP (CPDIS) and fat (FATDIS) were fitted to the following equation (Ørskov and McDonald, 1979):

DMDIS, CPDIS and FATDIS = $a + b (1-exp^{-c*t})$

where a = soluble fraction (% of total)

b = degradable fraction (% of total)

t = time of incubation (h)

c = rate of degradation.

This equation allows estimation of DM, CP and fat degradability at any incubation time, however, it does not predict effective degradability. The estimate of the fraction of DM or CP or fat that is actually degraded in the rumen, for a given rate of turnover from the rumen, is termed effective degradability. It is based on solubility (approximately equal to 0 time wash-out), rate of degradation and an estimated outflow rate from the rumen. The effective degradabilities for DM (EDDM), CP (EDCP) and fat (EDFAT) were calculated by the equation of Ørskov and McDonald (1979) as follows:

EDDM, EDCP and EDFAT = $a + ((b \times c)/(c + k))$

where a = soluble fraction (% of total),

b = degradable fraction (% of total)

c = fractional rate of degradation of degradable fraction

and k is 0.05 which is the value used to estimate the fractional rate of outflow from the rumen.

2.2.4. Statistical Analysis

Nylon bag data was analyzed for DM, CP and lipid disappearance using the procedure of GLM (general linear model) in SAS (1995). The replicated measure was time and the model included DM, CP, and fat disappearance. The Student-Newman Keuls (SNK) procedure was used to compare data means at a significance level of $P<0.05$. The nonlinear parameters a, b and c were estimated by the modified Marquardt method of SAS (1995) as discussed by Ørskov and McDonald (1979). The procedure used to analyze effective degradability of DM, CP and fat was GLM in SAS (1995). The model included cow and

treatment. The SNK procedure was used to compare data means at a significance level of $P<0.05$.

2.3 Results

2.3.1. Disappearance of Dry Matter, Crude Protein and Fat

The prime objective of the *in situ* nylon bag method was to determine the effect of formaldehyde and heat treated canola seed on ruminal disappearance of DM, CP and lipid.

Table 2.2. Effect of heat and formaldehyde treatments on dry matter, crude protein and crude fat disappearance of canola seed (%).

	Time of Incubation (h)					
Treatment	0.1	2	4	8	16	24
	Dry Matter (%)					
Untreated	12.31^{b}	20.18^{a}	23.72^{a}	40.01^{a}	73.70^{a}	81.42^{a}
Formaldehyde	7.32^{c}	11.06^{b}	14.37^{b}	24.90^{b}	33.0^{c}	40.53^{c}
Heat treated	17.01^{a}	21.43^{a}	25.77^{a}	36.83^{a}	61.66^{b}	72.41^{b}
SEM	0.57	1.48	0.54	1.66	2.98	1.16
	Crude Protein (%)					
Untreated	23.97^{a}	32.55^{a}	41.01^{a}	55.26^{a}	82.69^{a}	89.92^{a}
Formaldehyde	7.32^{b}	9.07^{b}	14.65^{b}	26.13^{b}	30.81^{b}	32.15^{b}
Heat treated	24.53^{a}	27.62^{a}	39.53^{a}	49.36^{a}	72.89^{a}	82.83^{a}
SEM	1.20	2.31	3.42	4.43	4.81	2.15
	Crude Fat (%)					
Untreated	9.44	15.19^{a}	18.92^{a}	32.62^{a}	73.83^{a}	85.74^{a}
Formaldehyde	0	4.71^{b}	8.81^{b}	11.30^{b}	17.63^{b}	24.05^{b}
Heat treated	13.27	18.24^{a}	20.13^{a}	29.41^{a}	59.22^{a}	76.98^{a}
SEM	0.70	2.16	2.46	2.52	4.30	1.72

^{a,b,c} Means in the same column with different superscripts differ ($P<0.05$)

The untreated canola seed acted as a control. The effect of ruminal disappearance of the three components is illustrated in Table 2.2. Dry matter disappearance for all the canola seed treatments increased with increasing rumen incubation time as expected. Dry matter disappearance of formaldehyde treated canola seed was lower than for the heat treated and untreated canola seed for all incubation times. There were no significant differences between the heat treated and untreated canola seed treatments for DM disappearance other than at 0.1, 16 and 24 h. At four hours of incubation, DM disappearance for the formaldehyde treated canola seed was 14.4% compared to 25.8 and 23.7% (SEM = 0.54) for heat treated and untreated canola seed, respectively. Figure 2.1 illustrates the DM degradation characteristics of the canola seed treatments used in the *in situ* study according to the equation of Ørskov and McDonald (1979). This figure clearly illustrates that with increasing incubation time, the untreated canola seed had the greatest percentage of disappearance, heat treated canola seed was similar to the untreated canola seed and the formaldehyde treated canola seed had the lowest percentage of ruminal disappearance.

Crude protein disappearance from the formaldehyde treated canola seed was significantly lower than observed for the heat treated and untreated canola seed at all incubation times. Disappearance of CP for the heat treated and untreated canola seed was not significantly different. At four hours of incubation, CP disappearance for the formaldehyde treated canola seed was 14.7% compared to 39.5 and 41.0% (SEM = 3.42) for heat treated and untreated canola seed, respectively. Figure 2.2 illustrates the CP degradation characteristics of the canola seed treatments according to the equation of Ørskov and McDonald (1979). This figure clearly indicates that CP disappearance with increasing incubation time was the greatest for the untreated canola seed compared to the formaldehyde treated canola seed. The difference between CP disappearance for the untreated and heat treated canola seed was not large (Table 2.2).

Fat disappearance from formaldehyde treated canola seed was significantly lower than observed for heat treated and untreated canola seed at all incubation times. There were no significant differences in fat disappearance for heat treated or untreated canola seed. Figure 2.3 indicates the fat degradation characteristics of the canola seed treatments. The difference

between fat disappearance of untreated and heat treated canola seed was not large. It was observed that formaldehyde treated canola seed had lower ruminal DM, CP and lipid disappearance in comparison to the heat treated and untreated canola seed. No significant effects on nutrient disappearance were observed with heat treatment of canola seed.

2.3.2. Degradation Characteristics For Dry Matter, Crude Protein and Fat

Table 2.3 illustrates the degradation characteristics of the soluble and degradable fractions, the rate of degradation and the effective degradability of DM, CP and fat. The soluble fraction of DM was not significantly different for the formaldehyde treated (8.7%) and untreated (8.8%) canola seed. Heat treated canola seed was significantly higher (13.7%) than the formaldehyde and heat treated canola seed (Table 2.3). The heat treated canola seed had the lowest degradable DM percentage and was significantly different from the other two treatments. The rate of DM degradation (%/h) was significantly different for the three canola seed treatments. The untreated canola seed had the highest rate of degradation (6.4%/h), heat treated canola seed was intermediate (4.6%/h) and formaldehyde treated canola seed had the lowest rate of degradation (1.9%/h). The effective DM degradability (EDDM) followed the same trend, being 60.0%, 55.2% and 33.8% for the untreated, heat treated, and formaldehyde treated canola seed, respectively. The degradation characteristics for CP indicated that the soluble and degradable fractions were not significantly different for the untreated and heat treated canola seed, however, the formaldehyde treated canola seed was significantly different from these two treatments. Formaldehyde treated canola seed was lower than the other two treatments for CP degradation and effective degradability. The rate of degradation (%/h) and the effective degradability of CP for these canola seed treatments was significantly different for each treatment, following the same pattern as seen with DM.

The degradability of the soluble fat fraction was significantly different for each treatment, being lowest for the formaldehyde treated canola seed and highest for the heat treated canola seed. Degradability of the degradable fat fraction for the different treatments was opposite that of the soluble fraction. The rate of degradation and the effective

degradability of fat followed the same pattern as with DM and CP.

Table 2.3. Effect of heat and formaldehyde treatment of canola seed on degradation characteristics and effective degradability of dry matter, crude protein and crude fat.

	Canola Seed Treatments			
	Untreated	Formaldehyde treated	Heat treated	SEM
Dry matter				
Soluble fraction, %	8.78[b]	8.66[b]	13.68[a]	0.45
Degradable fraction, %	91.22[a]	91.34[a]	86.32[b]	0.45
Rate of degradation, %/h	6.4[c]	1.9[a]	4.6[b]	0.002
EDDM[1]	60.04[a]	33.77[c]	55.16[b]	0.75
Crude protein				
Soluble fraction, %	20.79[a]	11.73[b]	21.37[a]	1.50
Degradable fraction, %	79.21[b]	88.20[a]	78.63[b]	1.48
Rate of degradation, %/h	8.3[a]	1.2[c]	6.2[b]	0.003
EDCP[2]	70.61[a]	29.07[c]	64.89[b]	1.20
Crude fat				
Soluble fraction, %	4.55[b]	1.27[c]	10.31[a]	0.51
Degradable fraction, %	94.46[b]	98.73[a]	89.69[c]	0.51
Rate of degradation, %/h	6.8[a]	1.2[c]	5.1[b]	0.002
EDFAT[3]	59.42[a]	20.83[c]	55.30[b]	0.93

[a,b,c] Means in the same row with different superscripts differ (P<0.05)
[1]EDDM= Effective degradability of dry matter assuming 5%/h outflow from rumen
[2]EDCP= Effective degradability of crude protein assuming 5%/h outflow from rumen
[3]EDFAT= Effective degradability of fat assuming 5%/h outflow from rumen

2.4. Discussion

2.4.1 Extent of Disappearance of Dry Matter, Crude Protein and Fat

Formaldehyde treated canola seed had slightly greater CP than the other canola seed

treatments. The higher CP in the formaldehyde treated canola seed may be attributed to the addition of full fat soybeans during the production of this product. The fat percentage was lowest for the formaldehyde treated canola seed compared to the other treatments. This lower fat content of formaldehyde treated canola seed may be attributed to the different seeds used to produce the formaldehyde product. It could also represent the dilution effect of adding full fat soybeans which have a lower fat content than canola seed. The fat content observed for the formaldehyde treated canola seed was confirmed by independent analysis conducted by Dr. John Ashes (1996).

The data compiled from the *in situ* nylon bag study demonstrated that treatment of canola seed by heat or formaldehyde influenced the rate and extent of disappearance of DM, CP and fat (Table 2.2). Untreated canola seed generally disappeared in the rumen more rapidly when compared to the heat treated and formaldehyde treated canola seed. Research by Khorasani et al. (1996, 1992), Deacon et al. (1988) and Kennelly et al. (1987) with Jet-Sploded® whole canola seed (high temperature for a short period of time), Protec® (a product based on formaldehyde treated whole canola seed and canola meal) and untreated whole canola seed illustrated that disappearance rates from nylon bags was the most rapid for untreated whole canola seed and lowest for the Jet-Sploded® whole canola seed and the Protec®. The present study also demonstrated that untreated canola seed had the greatest disappearance of DM and CP, heat treated canola seed was intermediate and formaldehyde treated canola seed was the lowest. Due to the low degradability, heat and formaldehyde treatment of canola seed should potentially increase the amount of CP and fat available for digestion in the small intestine.

2.4.2. Rate of Degradation Characteristics of Dry Matter, Crude Protein and Fat

Formaldehyde and heat treated canola seed treatments had lower effective degradabilites for DM and CP than the untreated canola seed which was similar to the findings of Deacon et al.(1988). In the current study, the formaldehyde treated canola seed had lower effective degradability for DM and CP than the heat treated canola seed. In the study by Deacon et al. (1988), the Jet-Sploded® canola had lower effective DM and CP

degradability than the Protec®, therefore, preparation of the treatment could have affected the effective degradability and disappearance rates. The data presented by Deacon et al. (1988) indicated that the rate and extent of DM and CP disappearance at 116°C was lower ($P<0.05$) than that observed for the untreated whole canola seed. The researchers also found that heat treatment of 154°C created the maximum effect for effective degradability of DM and CP. Heat treatment in the current study was 120°C for 15 min without addition of moisture. The current study confirms that treatment of canola seed either by heat or chemical treatment reduced ruminal disappearance and effective degradability. This is in agreement with Deacon et al. (1988) and Khorasani et al. (1992) and indicates that protection of canola seed reduces ruminal disappearance and effective degradability, therefore, increasing the amount of protein potentially available for digestion in the small intestine. The effect of heat on protection of nutrients is, however, temperature dependent as suggested by Deacon et al. (1988), thus, heat treatment from this study (120°C) was not as effective as heat treatment reported by Deacon et al. (1988) at 154°C.

The degradability of fat from the canola seed treatments reflects the degradation characteristics of DM and CP in the canola seed treatments. Thus, it is possible that the protection of the protein component of the canola seed resulted in increasing the amount of fat available for digestion in the small intestine.

2.5. Conclusion

The objective of this study was to investigate the effect of formaldehyde treated and heat treated canola seed on ruminal disappearance and degradation characteristics of DM, CP and fat and this was achieved. From the *in situ* nylon bag study it was concluded that protection of canola seed with formaldehyde treatment resulted in lower ruminal disappearance of DM, CP and fat compared to the heat treated and untreated canola seed. The degradation characteristics and effective degradabilities of these canola seed treatments also confirms that protecting canola seed from ruminal degradation reduces the rate of degradation and effective degradability of nutrients in canola seed.

Figure 2.1. Dry matter (DM) degradation characteristics of untreated, formaldehyde treated and heat treated canola seed.

DM degradation characteristics of canola seed

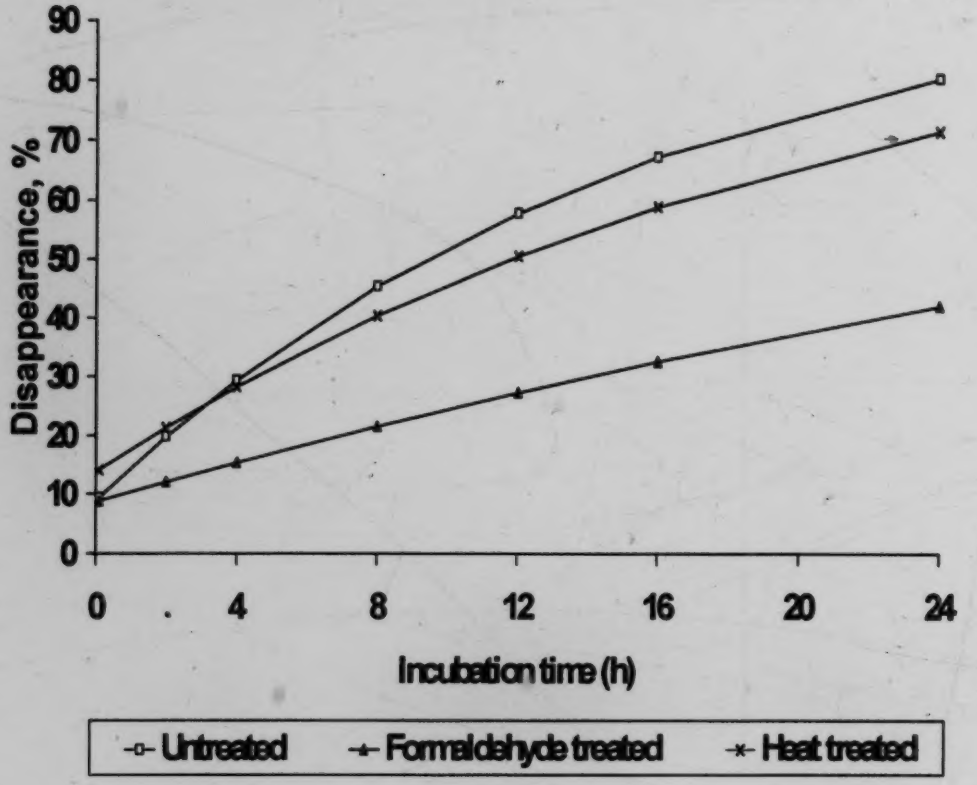


Figure 2.2. Crude protein (CP) degradation characteristics of untreated, formaldehyde treated and heat treated canola seed.

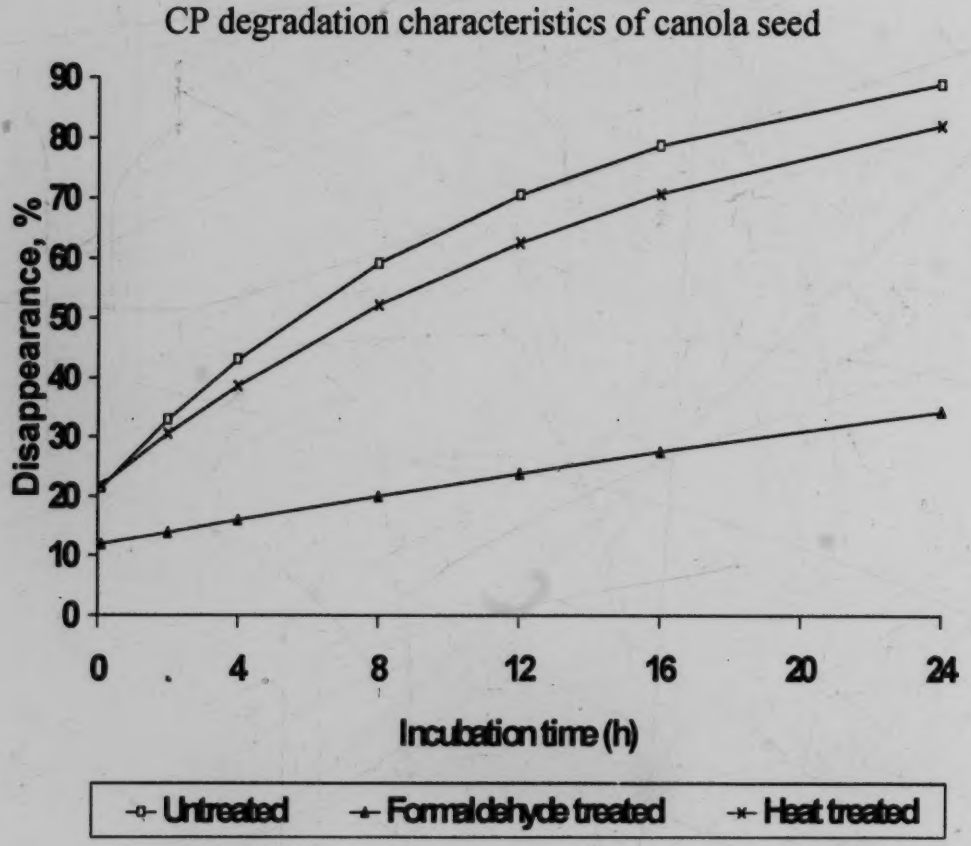
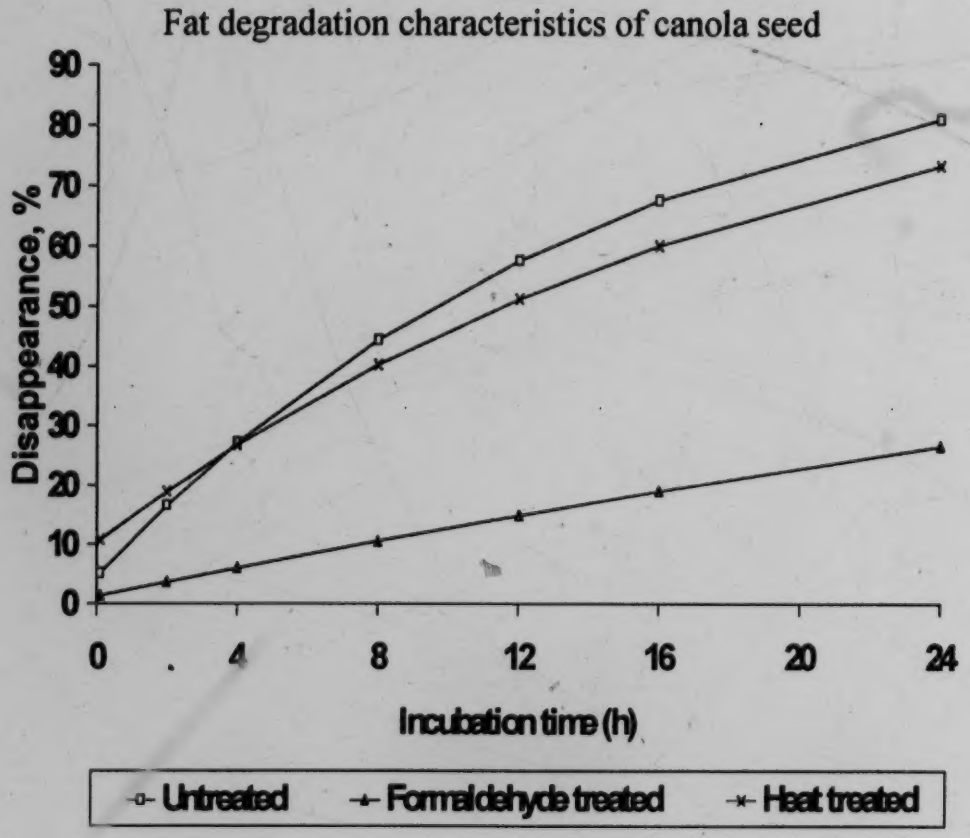

CP degradation characteristics of canola seed
Disappearance, %
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
Incubation time (h)
Untreated
Formaldehyde treated
Heat treated

Figure 2.3. Fat degradation characteristics of untreated, formaldehyde treated and heat treated canola seed.


Fat degradation characteristics of canola seed
Disappearance, %
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
Incubation time (h)
Untreated
Formaldehyde treated
Heat treated

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CHAPTER 3

Effect of Source of Dietary Fat on Ruminal and Whole Tract Digestion of Nutrients in Early-Lactation Holstein Cows.

3.1. Introduction

Dietary fat usually constitutes less than 3% of the ruminant diet and arises from forage, grains or seeds which are rich in linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) (Chilliard, 1993, Miller, 1979). During early-lactation dairy cows are in a state of negative energy balance and rely extensively on mobilization of long-chain fatty acids from adipose tissue for their energy requirements. High producing dairy cows in early-lactation are not able to consume sufficient amounts of feed to satisfy their energy requirements, therefore, producers are increasing the energy density of the diet by adding fat. Some fat sources are economically priced and can be used as a replacement for grain energy (Chilliard, 1993, Wu et al., 1991, Storry, 1981). Fat contains an energy value of about 2.25 times that of carbohydrates and proteins (Palmquist and Jenkins, 1980, Miller, 1979).

Canola seed contains approximately 40% fat which primarily consists of oleic (51%), linoleic (25%) and linolenic (14%) fatty acids (Khorasani et al., 1991). Palmquist and Jenkins (1980) and Devendra and Lewis (1974) indicate that the addition of dietary fat can adversely affect rumen fermentation and nutrient digestibility by physically coating the fibre and, thus, preventing microbial attack. Free fatty acids (FFA) can depress rumen microbial growth and the degree of saturation can influence rumen function and nutrient digestibility. The rumen population can be modified due to the toxic effects of fat on certain microorganisms (Palmquist and Jenkins, 1980). Due to these adverse effects there are limitations to how much dietary fat can be added to the ruminant diet. Generally up to 5% total added fat or 7-8% total dietary fat can be used without disruption of rumen function. The seed coat of canola seed acts as a barrier to protect the fat portion and is resistant to digestion both in the rumen and in the intestine. Processing of the canola seed such as cracking, grinding or rolling is necessary to achieve effective digestion of the seed (Kennelly, 1996, Khorasani et al., 1992). When canola seed is processed, the dietary inclusion level must be limited to avoid

detrimental effects on rumen function. In order to increase the inclusion of canola seed into the diet of the lactating dairy cow, the seed needs to be protected against microbial degradation. Protection is also necessary to prevent microbial biohydrogenation of fatty acids in the rumen.

Methods which have been used to protect fat supplements from rumen degradation include heat (Jet-Sploding® and extrusion) (Khorasani et al., 1992, Deacon et al., 1988) and chemical treatment (formaldehyde) (Ashes et al., 1995, 1992, Atwal et al., 1991). Treating the fat supplement with heat decreases the solubility of proteins, thereby decreasing their susceptibility to ruminal degradation (Deacon et al., 1986). Chemically treating (ie: formaldehyde) fat supplements results in encapsulation of the fat droplets in a layer of aldehyde treated protein which forms cross-linkages between the free amino groups making the supplement relatively insoluble and inert to bacterial breakdown in the rumen (Deacon et al., 1986, Storry et al., 1974). The protected fat supplement bypasses the rumen and under the acidic conditions of the abomasum, the protected supplement is released and made available for enzymatic digestion and absorption in the small intestine (Storry et al., 1974).

The hypothesis of the study was that heat and formaldehyde treatment of canola seed increases ruminal escape of unsaturated fatty acids to the intestine resulting in an increased supply of unsaturated fatty acids for incorporation into milk. Protection will also reduce the potentially negative effects of lipids on ruminal digestion. The objective of this study, therefore, was to investigate the effect of heat and formaldehyde treatment of canola seed on ruminal fermentation characteristics, nutrient flow to the intestine and total tract digestion of nutrients.

3.2. Materials and Methods

3.2.1. Animals and Experimental Design

Four mulitparious, ruminally and duodenally cannulated Holstein dairy cows in early-lactation (82 $\pm$ 20 DIM) were assigned to four treatments. The Holstein cows were fitted with a large rumen cannula (#1C-rumen cannula with rolled inner flange, 10 cm centre diameter, Bar Diamond Inc. Parma, ID) and a hard cannula (T-type) at the proximal

duodenum. The care of the dairy cows was in accordance with the Animal Use for Research or Teaching Protocol approved by the Animal Use Committee at the University of Alberta. Cows were randomly assigned to the four treatments in a 4 x 4 Latin square design (Table 3.1). An additional ruminally cannulated cow (82 DIM) in early-lactation was available and used for generating additional data on rumen fermentation characteristics of the animals. This shadow cow was assigned throughout the whole study with the same cow (Table 3.1).

Table 3.1. Latin square design and assignment of cows to treatments.

	Diet			
Period	Control	Untreated	Formaldehyde treated	Heat treated
1	8952	9074	9020(8820)	9125
2	9074	9020(8820)	9125	8952
3	9020(8820)	9125	8952	9074
4	9125	8952	9074	9020(8820)

Parentheses indicate shadow cow

Each experimental period was three weeks in length consisting of a change-over period for the first three days, an adaptation period from day 4 to 14 and a collection period from day 15 to 21. The change-over period involved increasing the new diet each day by 25% until day four when the new diet was fed at 100%. Cows were tethered in tie stalls and had free access to water.

3.2.2. Diets

A control diet was formulated to satisfy the nutrient requirements of a Holstein cow, weighing 600 kg, producing 40 kg milk per day of average composition (30 g of butterfat per kg milk), according to the NRC (1989). The control diet was comprised of 60% concentrate and 40% forage (alfalfa silage and barley silage as a 50:50 ratio) on a dry matter (DM) basis. The test concentrates supplied 1 kg/day (5% of diet on DM basis) of untreated, formaldehyde

Table 3.2. Formulation of concentrates (as fed basis) for the control, untreated canola seed, formaldehyde treated canola seed and heat treated canola seed diets (kg).

	Diet			
Ingredient	Control	Untreated	Formaldehyde treated	Heat treated
Barley grain	60.42	57.06	57.06	57.06
Canola meal	11.22	6.41	6.41	6.41
Corn grain-ground	10.08	10.08	10.08	10.08
Fish meal	1.57	1.57	1.57	1.57
Gluten meal	2.40	2.40	2.40	2.40
Meat and bone meal	4.07	4.07	4.07	4.07
Soybean	3.33	3.33	3.33	3.33
Cane molasses	3.53	3.53	3.53	3.53
Sodium bicarbonate	1.32	1.32	1.32	1.32
Salt - I	0.15	0.15	0.15	0.15
Fortified	0.62	0.62	0.62	0.62
Vitamin ADE	0.07	0.07	0.07	0.07
Vitamin D_3	0.01	0.01	0.01	0.01
Vitamin E	0.88	0.88	0.88	0.88
Mg Sulfate	0.10	0.10	0.10	0.10
Mg Oxide	0.10	0.10	0.10	0.10
Dynamate	0.12	0.12	0.12	0.12
Untreated canola seed (rolled)	-	8.16	-	-
Formaldehyde treated canola seed	-	-	8.16	-
Heat treated canola seed	-	-	-	8.16

For the control diet: CP, % DM 18.08; NEl, MCAL/kg 1.58; DIP, % of CP 66.64% supplied in 22 kg DM, 2.65 kg; UIP, % CP 33.36% supplied in 22kg DM, 1.33 kg; Ca, % DM 0.81; P, % DM 0.53; Vitamin E, IU/kg 46.08, supplies 1014.52 IU

For the test diets: CP, % DM 17.77; NEl, MCAL/kg 1.61; DIP, % of CP 66.53 % supplied in 22 kg DM, 2.60 kg; UIP, % of CP 33.47% supplied in 22 kg DM, 1.31 kg; Ca, % DM 0.82; P, % DM 0.55; Vitamin E, IU/kg 45.58, supplies 1003.57 IU.

treated and heat treated canola seed. Canola seed replaced canola meal and barley in the

control concentrate. Fat content of the canola seed was assumed to be 40% as indicated by research of Ashes et al. (1992). The concentrate mixtures were (1) control with no added fat (Control), (2) control + unprotected rolled canola seed, (3) control + formaldehyde protected canola seed and (4) control + heat treated canola seed. A total mixed ration (TMR) was fed *ad libitum* to all cows at 0800 h and 1800 h. The TMR was fed twice daily with two-thirds at 0800 h and one-third at 1800 h. Dry matter intake was recorded daily and adjusted to maintain 5 to 10% orts. Refusal amounts were recorded prior to each morning feeding. Table 3.3 shows the formulation of the TMR on a DM basis.

Table 3.3. Formulation of the total mixed ration (DM basis) for the dietary treatments (%).

	Diet			
Ingredient	Control	Untreated	Formaldehyde treated	Heat treated
Alfalfa silage	20.00	20.00	20.00	20.00
Barley silage	20.00	20.00	20.00	20.00
Concentrate 1	60.00	-	-	-
Concentrate 2	-	60.00	-	-
Concentrate 3	-	-	60.00	-
Concentrate 4	-	-	-	60.00

Concentrate 1 = no added fat
Concentrate 2 = untreated canola seed
Concentrate 3 = formaldehyde treated canola seed
Concentrate 4 = heat treated canola seed

3.2.3. Canola Seed Treatments

The formaldehyde treated canola seed was acquired from Dr. John Ashes of the Commonwealth Scientific Industrial Research Organization (CSIRO), Blacktown, New South Wales, Australia. The procedure for formaldehyde treatment of oilseeds involves computer aided control systems which mix the oilseed (dehulled) together with an antioxidant. The seed is then homogenized with water and alkali is introduced to solubilize the protein as

outlined by procedures of Scott and Ashes (1993) and Ashes et al. (1992). The formaldehyde treated canola seed consisted of 70% canola seed, 30% full fat soybeans and ethoxyquin (antioxidant) at 600 ppm. The untreated canola seed and the heat treated canola seed were both acquired from Mary Lou Swift of ProFORM Feeds Inc., Chilliwack, British Columbia, Canada. The untreated canola seed was rolled prior to feeding in the production study. The heat treated canola seed was heated at 120°C for 15 min. No moisture was added during the heating process.

3.2.4. Feed Sampling

Individual feed samples were collected on week three (collection week) each time a batch of feed was made, approximately every four days. The individual feed ingredients were composited to give one representative sample for the collection week; the composite sample was used for analysis. The orts were collected daily during the collection week and were composited into one sample at the end of week three for each period. Orts were collected prior to morning feeding and weighed within 30 min after collection. The test period orts and feed samples (week three) were frozen, composited and analyzed for DM, organic matter (OM), crude protein (CP) (Bradstreet, 1965), neutral detergent fibre (NDF), acid detergent fibre (ADF) (Van Soest et al., 1991) and ether extract (AOAC, 1990). The acid detergent residue was further treated with 72% sulphuric acid and ashed at 500°C to estimate cellulose and lignin, respectively. The DM was determined by drying at 110°C to a constant weight and OM was calculated as the difference between the DM and the residue after ashing at 500°C for at least 6 h. True DM was determined by using the following calculation:

True DM = (60°C DM sample (%) x 110°C DM sample (%))/100.

All feed components (DM, OM, CP, NDF, ADF, lignin, hemicellulose, cellulose, ether extract) were based on their percentage in the TMR and were on a DM basis. The proportion of the individual ingredients (ie: alfalfa silage, barley silage, concentrate) in the TMR, therefore, was multiplied by its DM and the individual analysis for each component and

divided by its proportion multiplied by its DM. All feed and orts samples were ground to pass a 1 mm screen (Thomas-Wiley Laboratory Mill, Model 4, Arthur H. Thomas Co., Philadelphia, PA, USA) prior to analysis. All of the feed samples were analyzed in duplicate.

3.2.5. Rumen Fermentation

Rumen liquid was sampled over a 24 h period during the collection week on day 18 beginning at 0750 h for pH, lactic acid, ammonia and VFA and ending at 0750 h on day 19. Collection of rumen fluid for pH and VFA were at the following times: 0750, 0830, 0900, 0930, 1000, 1100, 1200, 1400, 1750, 1830, 1900, 1930, 2000, 2200 h (day 18), 0030, 0200, 0600 and 0750 h (day 19). Thus, a total of 18 samples per cow per period for rumen pH and VFA. The collection of rumen fluid for lactic acid was at the following times: 0750, 0830, 0900, 0930, 1100, 1200, 1400, 1750, 1830, 1900, 1930, 2000, 2200 h (day 18), 0200, 0600 and 0750 h (day 19). Thus, a total of 16 samples per cow per period for lactic acid. Collection of rumen fluid for ammonia was at the following times 0750, 0900, 1000, 1100, 1200, 1400, 1750, 1900, 2000, 2200 h (day 18), 0030, 0200, 0600 and 0750 h (day 19). Thus, a total of 14 samples per cow per period for rumen ammonia. Rumen fluid was collected within 5 min of the above listed sampling times for pH, VFA, lactic acid and ammonia. Rumen fluid was sampled using a stainless steel strainer attached to a plastic tube which was inserted into the rumen. Rumen liquid was extracted by applying a vacuum to the end of the tube with a syringe. The amount of rumen fluid taken at each collection time varied between 100 and 200 ml. After collection, rumen fluid (4 ml) was added to test tubes with prepared reagents (1 ml of 25% ortho-phosphoric acid for the VFA tubes), frozen immediately and stored at -30°C until further analysis.

Rumen pH was recorded using a calibrated pH meter. Analysis for lactic acid in rumen fluid was according to the procedures outlined by Khorasani et al. (1996) with modifications outlined in Appendix 3.1. Volatile fatty acid analysis in rumen fluid was according to Khorasani et al. (1996) and Erwin et al. (1961) and modifications are outlined in Appendix 3.2. Ammonia N analysis was according to Fawcett and Scott (1960) and involved preparing the following reagents: sodium phenate, sodium nitropurside, sodium

hypochlorite and a standard solution (100 μl NH_3-N/ml). Rumen fluid and duodenal digesta were analyzed for ammonia N which is outlined in Appendix 3.3.

3.2.6. Forestomach Digestibility

Chromium (Cr)-mordanted alfalfa hay was used as a solid phase digestibility marker. The Cr marked alfalfa was prepared using the procedure of Uden et al. (1980) with modifications outlined in Appendix 3.4. The amount of marker (Cr-mordanted alfalfa) added to the rumen contents was 30 g per day per cow. Chromium marked alfalfa was weighed onto filter papers and the papers were manually placed into the rumen at intervals starting on day 12 of each period. The marker was placed into the rumen at 0700, 1330 and 2100 h from day 12 to 16 inclusive and at 0500, 1100, 1700 and 2300 h from day 17 to 20 inclusive to achieve steady-state concentrations in duodenal digesta and faeces. Marker dose was within 15 min of the listed time.

Duodenal digesta samples were collected on five occasions per 72 h starting from 1030 h on day 17 for each period. The other collection times were 0548 (day 18), 0100 and 2012 (day 19) and 1524 h (day 20). Duodenal sample collection was within 15 min prior to the listed collection time. In order to collect duodenal digesta, the duodenal plug was removed and a stainless steel gate connected to plastic tubing was inserted into the cannula resulting in total collection of duodenal contents. The duodenal contents were allowed to flow through the gate and plastic tubing into a plastic bucket. Approximately two litres of duodenal digesta were collected on each occasion, mixed well and approximately 250 ml were taken from each collection and frozen for determination of DM, OM, CP, diaminopimelic acid (DAPA) concentration and Cr-marker determination. About 70 - 80 ml of duodenal digesta were taken and frozen for CP (fresh) and ammonia N determination. All duodenal digesta collected was frozen immediately and pooled for each period. Duodenal samples which were collected for DM, OM, CP and Cr-marker were dried in a 60°C forced air oven for 72 h and weighed 24 h after removing from the oven. Dried samples were ground to pass a 1 mm screen (Thomas-Wiley Laboratory Mill, Model 4, Arthur H. Thomas Co., Philadelphia, PA, USA). Solid phase digestibility was estimated by the Cr marker (Khorasani et al., 1993a) as

outlined in Appendix 3.5.

3.2.7. Rumen Bacterial Yield

Daily duodenal flow of bacterial N and OM was estimated from the DAPA concentration of duodenal digesta matter and the N/DAPA ratios of isolated rumen bacteria. One litre of rumen fluid was collected at 0700, 1300 and 2000 h on day 18 of each period for bacterial isolation. Samples were collected within 5 min of the listed collection time. The isolation procedure for rumen bacteria was according to Robinson and Sniffen (1985) and modifications are outlined in Appendix 3.6. All the bacterial samples were pooled for each period. Samples were analyzed for DM, OM, CP (AOAC, 1984), DAPA and N. Diaminopimelic acid and N were determined by the procedure of Russell and Robinson (1984).

3.2.8. Whole Tract Digestion

Faeces were collected by rectal grab sampling five times over the 72 h intensive study period from 1030 h on day 17 of each period. Faecal samples were collected at 1030 (day 17), 0548 (day 18), 0100 and 2012 (day 19) and 1524 h (day 20). Faecal sample collection was within 15 min prior to the listed collection time. On each occasion 400 g of faeces was collected by rectal grab sampling failing voluntary defecation from each cow. Faecal samples were frozen immediately and pooled at the end of each collection period. At the end of the collection period, faecal samples were thawed and 1 kg of mixed sample was oven dried at 60°C for 72 h, allowed to cool for 24 h and weighed. The faecal samples were ground to pass a 1 mm screen (Thomas-Wiley Laboratory Mill, Model 4, Arthur H. Thomas Co., Philadelphia, PA, USA) for proximate analysis and Cr- marker determination. The digestibility was estimated using the marker calculation for Cr-marker. True DM was determined using the calculation for true DM as for feed samples and digesta.

3.2.9. Statistical Analysis

The data was analyzed as a Latin square design using a model which included cow,

period and diet. The procedure of general linear models (GLM) in SAS (1995) was used to analyze the data for feed intake, forestomach (duodenal) and whole tract (faecal) digestibility, duodenal N fractions and bacterial composition and yield. The procedure of Univariate Normal was used to analyze the diets for DM, OM, NDF, ADF, lignin, hemicellulose, cellulose and ether extract. The rumen metabolites (ammonia N, lactate and VFA) and rumen pH were analyzed as repeated measure by the GLM procedure in SAS (1995). The model included cow, period, diet and the interactions time x cow, time x period, time x diet. The error term was the interaction of cow x period x time. The data least square means (lsmeans) were compared at a significance level of $P<0.05$ using pdiff.

3.3. Results

3.3.1. Animals and Experimental Design

The cow which was assigned a shadow cow at the start of the experiment completed the study. The data from the shadow cow was analyzed as for the initial cow. No duodenal or bacterial data was obtained from the shadow cow since she was not cannulated at the duodenum. One cow (cow 9125) completed the first period of the experiment but did not complete the remaining periods (Table 3.1). The loss of this cow was the result of health problems not related to this study. The data collected from this animal was not used for any of the statistical analysis. The loss of this cow resulted in the experiment having three cows (with one shadow cow) and four diets, therefore, the lsmeans of the data are presented in the tables.

3.3.2. Dry Matter Intake

The DM, OM, CP, NDF, ADF, lignin, hemicellulose, cellulose and ether extract concentrations for the four dietary treatments are presented in Table 3.4. These components were found to be quite similar for the dietary treatments and were not influenced by treatment. No significant treatment differences were found for DM intake (Table 3.5). Although animals fed the untreated canola seed had numerically higher DM intake, no significant differences were found among the dietary treatments. The intakes (Table 3.5) for

OM, CP, NDF, ADF, hemicellulose and cellulose were also not significantly different for the four dietary treatments.

Table 3.4. Least square means for chemical composition of the dietary treatments (%).

Item	Diet				
	Control	Untreated	Formaldehyde treated	Heat treated	SEM
Dry Matter	46.3	47.3	47.2	49.7	3.03
Crude Protein	21.3	20.7	21.6	20.4	0.87
Organic Matter	90.6	90.7	90.1	90.3	0.47
NDF	33.2	32.6	33.3	34.8	2.41
ADF	15.9	15.9	15.8	16.1	0.86
Lignin	3.1	2.9	3.0	2.9	0.33
Hemicellulose	17.4	16.7	17.5	18.8	1.92
Cellulose	12.8	13.0	12.9	13.2	0.64
Ether Extract	2.8	4.8	4.1	4.3	0.34

3.3.3. Apparent Forestomach and Whole Tract Digestibility

Apparent forestomach and whole tract digestibilities are in shown Table 3.5. In general, cows fed the formaldehyde treated canola seed diet had the lowest apparent forestomach digestion coefficients. Whole tract digestion was similar for cows fed the heat treated and untreated canola seed treatments for DM, OM, CP, NDF, ADF, hemicellulose and cellulose. Forestomach digestibility of DM, OM and CP was not significantly different for the dietary treatments. Dry matter and OM digestibility at the forestomach tended to be higher for cows fed the heat treated canola seed diet in comparison to the formaldehyde treated canola seed diet ($P = 0.07$). Significant treatment differences in apparent forestomach digestibility were observed for NDF, ADF, hemicellulose and cellulose. Cows fed the control diet were similar to cows fed the untreated and heat treated canola seed diets for NDF, ADF, hemicellulose and cellulose forestomach digestibility. Cows fed formaldehyde treated canola

Table 3.5. Least squares means for dry matter intake(kg/d) and apparent forestomach and whole tract digestion (%).

Parameter	Diet				SEM
	Control	Untreated	Formaldehyde treated	Heat treated	
Dry Matter					
Intake, kg/d	20.29	22.76	20.67	20.33	0.97
Digestion, %					
Forestomach	53.25	49.97	37.97	53.81	4.03
Whole tract	74.38^{a}	71.92^{b}	72.85^{ab}	71.86^{b}	0.56
Organic Matter					
Intake, kg/d	18.44	20.59	18.70	18.32	0.87
Digestion, %					
Forestomach	48.58	44.83	31.00	48.93	4.50
Whole tract	71.83^{a}	69.01^{b}	69.94^{ab}	68.73^{b}	0.60
Crude Protein					
Intake, kg/d	4.58	5.40	5.04	4.74	0.26
Digestion, %					
Forestomach	46.96	47.33	32.20	43.23	5.64
Whole tract	79.57	77.76	76.18	77.24	1.71
NDF					
Intake, kg/d	6.87	7.48	6.95	6.68	0.44
Digestion, %					
Forestomach	54.25^{a}	47.25^{ab}	40.48^{b}	56.25^{a}	2.58
Whole tract	60.50^{a}	55.36^{b}	55.97^{b}	56.17^{b}	1.08
ADF					
Intake, kg/d	3.19	3.65	3.29	3.13	0.16
Digestion, %					
Forestomach	47.12^{ab}	41.06^{ab}	30.03^{b}	49.72^{a}	4.26
Whole tract	53.36^{a}	50.87^{ab}	49.24^{b}	48.54^{b}	0.97
Hemicellulose					
Intake, kg/d	3.69	3.82	3.66	3.56	0.29
Digestion, %					
Forestomach	60.24^{ab}	53.34^{bc}	49.46^{c}	62.26^{a}	1.66
Whole tract	65.90	59.71	60.40	62.49	2.09
Cellulose					
Intake, kg/d	2.60	2.98	2.67	2.57	0.14
Digestion, %					
Forestomach	54.06^{a}	47.62^{ab}	40.32^{b}	56.05^{a}	3.09
Whole tract	61.80^{a}	57.97^{ab}	57.30^{b}	56.31^{b}	1.23

^{a,b,c} Means in the same row with different superscripts differ ($P<0.05$)

seed diet was significantly ($P<0.05$) lower from the control and heat treated canola seed fed cows for NDF, hemicellulose and cellulose forestomach digestibility (Table 3.5). No significant treatment differences were noted for whole tract digestibility of CP and hemicellulose among treatments. Cows fed the control diet had significantly greater NDF whole tract digestibility than cows fed the other treatments. The control diet was significantly greater than the formaldehyde treated and heat treated canola seed diets for whole tract ADF digestion. Whole tract digestibility of NDF, ADF and cellulose were not significantly different for cows fed the canola seed diets but were significantly lower when compared to the control fed cows.

3.3.4. Rumen Fermentation

The mean ruminal pH was not significantly different for the dietary treatments (Table 3.6). Figure 3.1 illustrates the diurnal patterns of ruminal pH over the 24 h feeding period. Time of morning feeding was approximately 0800 h and the first collection of rumen fluid was at 0750 h. After feeding (0800 h), rumen pH declined for all treatments within a short period of time (30 min after feeding) and then increased except for the formaldehyde treated canola seed which declined slightly further. Similar values for rumen pH were recorded at both 0750 h sampling times. No significant differences were found for mean rumen lactate (Table 3.6). Figure 3.2 illustrates the diurnal pattern of rumen lactic acid for the four dietary treatments over the 24 h collection period. The mean rumen concentration of ammonia N was not significantly different for the four dietary treatments (Table 3.6). Figure 3.3 illustrates the diurnal pattern of rumen ammonia N over the 24 h collection period with 14 collection times (14 samples per cow). All diets utilized in this study had ammonia concentrations above 5 mg/100 ml (5 mg/dl).

No statistical differences were observed for the concentration of total rumen VFA in rumen fluid (Table 3.6). Figure 3.4 depicts the diurnal pattern of the total VFA in rumen fluid over the 24 h collection period. The concentration of individual VFA was not influenced by diet (Table 3.6). The diurnal pattern of acetic acid for the dietary treatments is displayed in Figure 3.5 for the 24 h collection period. Similar values for the percentage of acetic acid

Table 3.6. Least square means for rumen fermentation characteristics for cows fed the dietary treatments.

Component	Diet				SEM
	Control	Untreated	Formaldehyde treated	Heat treated	
Ruminal pH	6.28	6.18	6.27	6.24	0.05
Lactate, m*M*	18.79	25.44	20.79	19.01	3.26
NH_3N, mg/100 ml	18.63	19.04	16.69	17.79	1.26
Total VFA, m*M*	127.77	128.46	128.57	121.38	6.24
VFA Concentration, mol/100ml					
Acetate	59.26	57.19	59.70	57.90	1.27
Propionate	23.54	25.53	23.82	24.97	1.64
Isobutyrate	1.10	0.98	1.02	1.05	0.04
Butyrate	12.10	12.34	11.62	11.97	0.33
Isovalerate	1.72	1.48	1.60	1.75	0.15
Valerate	1.83	2.00	1.81	1.91	0.07
Caproate	0.44	0.48	0.44	0.48	0.05
Branch-chain VFA[1]	2.82	2.46	2.62	2.79	0.18
Acetate:Propionate	2.58	2.30	2.51	2.46	0.26

[1]Branch-chain VFA = isobutyric + isovaleric acids

found in rumen fluid were observed for both 0750 h sampling times. Figure 3.6 illustrates the diurnal pattern of propionic acid for the four dietary treatments during the 24 h collection period. Isobutyrate and butyrate concentrations for the dietary treatments were also not influenced by dietary treatment. The percentage of isobutyric acid and butyric acid in rumen fluid over a 24 h collection period are illustrated in Figure 3.7 and Figure 3.8, respectively. The concentration of isovalerate and valerate in rumen fluid did not exhibit any significant treatment differences (Table 3.6). Figure 3.9 and Figure 3.10 illustrate the diurnal pattern of isovaleric acid and valeric acid, respectively, for the four dietary treatments. Figure 3.11

depicts the diurnal pattern of the branch-chain VFA for the 24 h collection of rumen fluid. No treatment differences were observed for caproic acid concentration in rumen fluid (Table 3.6). Figure 3.12 illustrates the diurnal pattern of caproate for the 24 h collection period. No significant differences were found for the acetate:propionate ratio for the dietary treatments (Table 3.6). Figure 3.13 illustrates the diurnal pattern for the acetate:propionate ratio for the collection times listed.

3.3.5. Duodenal Nitrogen Fractions and Rumen Bacterial Yield and Composition

The effects of dietary treatments on duodenal N fractions, ruminal bacterial composition and yield and efficiency of bacterial N capture is shown in Table 3.7. For N intake, total N and ammonia N flow at the duodenum, the control tended to be lower ($P>0.10$) than the canola seed treatments. No significant treatment differences ($P>0.05$) were observed for N intake, duodenal N flow or non-ammonia N for dietary treatments. Dietary treatment did not affect ($P>0.05$) the total bacterial N (% OM) flow to the duodenum or bacterial N as a percentage of N intake. Diaminopimelic acid (DAPA) content of bacteria (mg/g of OM) was not significantly affected by the dietary treatments. Diaminopimelic acid (mg/g of OM) of bacteria sampled from cows fed the heat treated canola seed diet, however, tended to be higher than the DAPA content of bacteria sampled from cows fed the formaldehyde treated canola seed ($P = 0.07$).

Bacterial N expressed as grams per millimole (mmol) of DAPA and bacterial N production (g/day) were not significantly different for the dietary treatments. When bacterial N is expressed as a percentage of non-ammonia N, no significant dietary treatment effects were observed. Residual N was calculated by subtracting bacterial N and ammonia N flow at the duodenum from the total N flow at the duodenum (Table 3.7). Residual N, therefore, consists of rumen undegradable protein (UIP), protozoa and endogenous protein. No significant differences occurred for residual N or when expressed as a percentage of N intake within the dietary treatments.

Efficiency of microbial protein synthesis is most commonly expressed as grams of bacterial N per kilogram of OM apparently digested in the rumen (ADOM). Microbial

Table 3.7. Least square means of duodenal nitrogen fractions, including ruminal bacterial composition and yield, as influenced by cows fed the dietary treatments.

	Diet				
Parameter	Control	Untreated	Formaldehyde treated	Heat treated	SEM
N Intake, g/d	637.27	798.07	722.87	647.67	51.70
Duodenal N flow, g/d					
Total N	336.75	410.76	427.91	435.42	69.31
NH_3 N	3.25	4.99	3.54	7.30	3.53
Non NH_3 N	333.50	405.77	424.36	428.13	65.81
Percentage of N intake	52.55	52.14	56.36	67.01	5.28
Bacterial					
Total N, % OM	10.11	10.44	10.62	10.48	0.19
DAPA[1], mg/g of OM	5.14	5.73	5.36	6.38	0.32
N, g/mmol of DAPA	3.85	3.51	3.76	3.19	0.19
N, g/d	264.16	283.26	297.15	270.11	29.65
N as % N intake	41.70	36.09	38.75	38.30	3.13
N as % non-NH_3 N	79.15	69.37	71.85	57.08	8.02
Residual N, g/d	69.33	122.51	127.21	158.02	80.00
Percentage of N intake	10.86	16.05	17.61	28.71	6.49
Ruminal bacterial yield N, g/kg of ADOM[2]	28.75	31.59	29.23	41.00	5.56
Protein Capture[3], %	46.33	43.31	47.71	54.09	3.87

[1]Diaminopimelic acid,[2]Apparently digested OM in the rumen,[3]Protein captured = 100 x (bacterial CP)/(CP intake - residual).

efficiency per unit of OM apparently digested in the rumen was not significantly different for the dietary treatments. When efficiency of bacterial N capture was calculated based on actual bacterial CP yield and actual CP intake, the actual efficiency of bacterial N capture was not significantly affected by the dietary treatments ($P>0.05$).

3.4. Discussion

3.4.1. Dry Matter Intake

The nutrient analysis of the diet was not influenced by treatment (Table 3.5). The

control diet was formulated to have 18.0% CP (Table 3.2), however, the actual CP percentage of the control was 21.3% (% DM) (Table 3.4). The test diets (canola seed treatments) were formulated to contain 17.8% CP (Table 3.2), however, the actual CP content of the test diets was higher (Table 3.4).

Dry matter intakes were not influenced by dietary treatment which indicates that the type of canola seed treatment did not influence feeding habits of the dairy cow. Both the formaldehyde treated and heat treated canola seed had distinct aromas. The heat treated canola seed had a sweet roasted smell and the formaldehyde treated canola seed had a somewhat strong odour of formaldehyde. The untreated canola seed did not have a distinct odour. Other researchers, such as Khorasani and Kennelly (1996, 1995), Ashes et al. (1992) and Khorasani et al. (1991), who fed either heat treated or formaldehyde treated canola seed did not observe any influence on DM intake for the dairy cows in early-lactation. Research by Atwal et al. (1991), however, found that by feeding formaldehyde treated canola seed (approximately 850 g of oil/day) to mid-lactation cows DM intake decreased. Schauff and Clark (1992) reported that DM intake of cows fed supplemental fat greater than 3% of the diet decreased DM intake. The amount of fat fed in the current study was approximately 2.1% and did not influence DM intake.

The apparent discrepancies in the literature on the effect of dietary fat on DM intake can be related to fat source, method of processing, level of inclusion, stage of lactation, cow variation and/or experimental design. Palmquist (1990) hypothesized that during periods of rapid adipose mobilization (early-lactation), cows may decrease feed intake to regulate fatty acid concentration in plasma. Palmquist and Conard (1978) demonstrated that when fatty acids are protected from rumen biohydrogenation by heat or chemical treatment they could be included at higher concentrations in the diet without apparently influencing intake. At an inclusion level of 5% canola seed in the diet (DM basis), protected or unprotected canola seed did not influence intake of DM, OM, CP, NDF, ADF, hemicellulose or cellulose. In summary, DM intake and its components were not influenced by the inclusion of the canola seed in the diet.

3.4.2. Forestomach and Whole Tract Digestibility

Digestibility of a feedstuff is measured as the proportion of the feed which is not excreted in the faeces and is, therefore, assumed to be absorbed by the animal (McDonald et al., 1988). The formaldehyde treated canola seed diet exhibited numerically lower percentages for forestomach digestibility of DM and its components indicating greater ruminal escape than observed for the other treatments. This is in agreement with the *in situ* study (Chapter 2) which found that ruminal disappearance and degradation for DM, CP and fat was lowest for the formaldehyde treated canola seed. In this digestibility study, there were no significant differences between the heat treated and untreated canola seed treatments.

Khorasani et al. (1992) observed no significant treatment differences for the apparent whole tract digestibility coefficients for DM, CP, NDF and ADF at 4.5 and 9% inclusion levels of heat treated canola seed, however, a trend for lower digestibility was observed at 13.2 and 17.4% inclusion of heat treated canola seed. The authors indicated that the benefits of increased energy density associated with fat supplementation may have been progressively lost with increasing fat addition to the diet. Murphy et al. (1987) investigated total diet digestibilites of lactating dairy cows fed full fat rapeseed at either 1 kg or 2 kg/day. In this 1987 study, there was a treatment effect on DM and NDF digested in the hindgut with increased rapeseed feeding ($P<0.05$). Elliott et al. (1994), Palmquist (1991) and Grummer (1988) also found that fat supplementation had limited effects on total tract digestibility.

3.4.3. Rumen pH

There are a number of factors which can affect rumen pH such as (1) feed intake level, (2) physical form of the feedstuff, (3) dietary buffering compounds, (4) concentrations of rapidly fermentable carbohydrates, (5) feeding patterns and (6) a decrease in fibre digestion by increasing the rate of passage caused by increased intake (Mertens, 1979). No significant differences were found in rumen fermentation characteristics for the dietary treatments. Fluctuations in rumen pH would be expected throughout the day depending on feeding habits of the dairy cow. Murphy et al. (1987) found no differences between night and day pH values and rumen pH only slightly increased three hours after feeding. The general range for rumen

pH is within 5.8 to 6.8 (Mertens, 1979, Harfoot, 1978) which was found in this study. The lack of effect of dietary fat on average ruminal pH is in agreement with research by Khorasani and Kennelly (1996), Teh et al. (1994) and Drackley and Elliott (1993) who studied various protection methods for supplemental fat at inclusion levels ranging between 3 and 6% of dietary DM.

3.4.4. Rumen Lactic Acid

The concentrations of lactic acid and VFA are useful in assessing the extent and nature of microbial fermentation. Lactic acid is one of the intermediate compounds in the fermentation of starch to propionic acid. Kennelly (1996) and Bath (1982) describe lactic acid production in the ruminant animal. If large amounts of readily fermentable carbohydrates, such as starch from grain, are consumed by a dairy cow beyond the fermentable capacity of the microorganisms in the rumen, glucose accumulates in the rumen and can lead to rapid growth of lactic acid bacteria.

Lactic acid concentrations were not significantly different among the dietary treatments. Khorasani et al. (1996) indicates that ruminal concentrations of lactate fluctuate throughout the day and lactate concentrations are higher after feeding than prior to feeding. In the present study, lactic acid concentrations fluctuated throughout the day and were higher after feeding and were lowest prior to the 1800 and 0800 h feeding times.

3.4.5. Rumen Ammonia Nitrogen

The concentration of rumen ammonia at any one time is the result of many simultaneous factors. Obara et al. (1991) indicated that rumen concentrations of ammonia N are positively related to N intake. This is in agreement with Robinson et al. (1991) who also stated that diets with more fermentable forms of N are associated with higher average concentrations and/or peak concentrations of ammonia than are diets containing N sources more resistant to rumen proteolysis. Khorasani et al. (1996) indicates that the contribution of dietary non-ammonia N to ruminal ammonia N was dependent on the N content of the diet and solubility and degradability of the dietary protein. Rumen ammonia concentrations,

therefore, are the net result of rates of utilization and production by rumen microorganisms as well as absorption from the rumen and passage with rumen contents to the abomasum (Robinson et al., 1991).

Average rumen concentrations of ammonia N were not significantly different for the dietary treatments. The formaldehyde treated and heat treated canola seed diets, however, had numerically lower concentrations of ammonia in comparison to the control and the untreated canola seed diets. The absence of significant differences in ammonia N is also consistent with research by Khorasani and Kennelly (1996), Elliott et al. (1993) and Sharma et al. (1978) who utilized fat sources at inclusion levels of 5%. As indicated by Figure 3.3, the concentrations of ammonia N were greater than 5 mg/dl and the average rumen ammonia N concentration was greater than 5 mg/dl at all sampling times. Satter and Slyter (1974) indicated that rumen ammonia N concentrations above 5 mg/dl are required to maximize growth of ruminal bacteria. Ørskov (1982) stated that ammonia concentrations show a marked diurnal variation associated with feeding times. The rumen concentrations of ammonia N for the present experiment were also lower prior to feeding and increased after feeding. Finn et al. (1985) and Russell et al. (1981) reported increases in ammonia concentrations were the greatest one to three hours after ingestion of a meal. For this study, rumen ammonia concentrations increased approximately two hours postfeeding.

3.4.6. Rumen Volatile Fatty Acids

Volatile fatty acids are the major source of energy absorbed from the digestive tract in the ruminant and are the main end products of anaerobic fermentation of carbohydrates. The principle VFA which are utilized for energy production are acetate, propionate and butyrate and are important building blocks for milk fat and solids-not-fat (SNF) in milk. The VFA that have the greatest effect on milk fat synthesis are acetic and propionic acids. The concentration of total VFA in rumen fluid ranges from approximately 20 m*M* to over 100 m*M* (Harfoot, 1978). For this study the total VFA concentration was approximately 95 to 129 m*M*. Characteristics of rumen fermentation, in terms of total VFA, when cows consumed diets containing added canola seed were not significantly different ($P>0.05$) from those cows

which consumed the control diet. Palmquist and Jenkins (1980) have indicated that ruminal VFA concentrations are usually not affected by dietary fat additions, however, some fat sources can interfere with rumen fermentation resulting in a decline in total VFA production (Khorasani and Kennelly, 1996, Khorasani et al., 1992, Elliott et al., 1994). Kim et al. (1993) suggests that lower total VFA concentrations with high fat diets may indicate some interference with rumen microbial activity or reduced availability of rumen fermentable carbohydrates. Since no significant differences were found in this study for total VFA concentrations, this would illustrate that the inclusion level of the unprotected and protected canola seed diets did not influence rumen VFA characteristics possibly due to a low inclusion level of dietary fat (approximately 2.1%). Markus et al. (1996), Palmquist (1991) and Grummer (1988) also found no significant differences among treatments in the concentration of total rumen VFA.

Individual VFA production and the acetate:propionate ratio for the four dietary treatments were not significantly different. The average concentrations and diurnal patterns of individual VFA and the acetate:propionate ratio did show some numerical differences, however, these differences were relatively small and could be related to animal variation. Acetic acid is considered one of the main building components of milk fat (Bath, 1982). As previously stated, acetic acid was not influenced by the inclusion of dietary fat and comprised approximately 60% of the total VFA. This is in agreement with a study by Khorasani and Kennelly (1996) in which 3.75% (DM basis) heat treated canola seed was included in the diet. These authors indicated that since there were no significant differences in overall means of rumen acetate it is possible the microbial fermentation was not affected by the heat treated canola seed. Khorasani et al. (1993b), however, observed that rumen acetate concentration decreased linearly with increasing dietary inclusion of heat treated canola seed.

Propionic acid is required for a variety of metabolic functions which include lactose synthesis, energy for metabolic functions and when in excess, for body fat deposition (Bath, 1982). Propionic acid concentrations were approximately 25% of the total VFA and did not exhibit any significant differences among the treatments. This finding is in agreement with Markus et al. (1996), Kim et al. (1993) and Palmquist (1991). Other researchers, however,

have observed that propionate concentrations declined with the inclusion of dietary fat at levels greater than approximately 5% of dietary DM (Khorasani and Kennelly, 1996, Khorasani et al., 1992). Khorasani and Kennelly (1996) and Khorasani et al. (1992) stated that added dietary fat replaces nonstructural carbohydrates, lowers the availability of fermentable carbohydrates for VFA production causing a decrease in total VFA production as well as propionate production. No significant differences in acetate and propionate concentrations or in the acetate:propionate ratio suggest that the cows maintained normal rumen fermentation patterns and digestion. Khorasani et al. (1992) stated that the absence of significant differences in the acetate:propionate ratio and the total VFA concentration suggests that the dietary treatments were similar in fermentability. The data for this study is also in agreement with research by Kim et al. (1993) and Drackley and Elliott (1993) who also observed no effect of 4% (of DM) added dietary fat on the acetate:propionate ratio.

Butyrate is another important VFA which is a precursor for β-hydroxybutyrate and milk fat. As previously stated, no significant differences in butyrate or isobutyrate concentrations were observed. Research on the addition of dietary fat to lactating dairy cow diets found the concentration of butyrate and isobutyrate were not significantly different for the dietary treatments (Khorasani and Kennelly, 1996, Khorasani et al., 1992, Elliott et al., 1993). Khorasani and Kennelly (1996) indicated that since no significant differences in the overall means of butyrate and isobutyrate were observed, it is possible that microbial fermentation was not affected by heat treated canola seed at an inclusion level of 3.75% (of DM). In the present study, the isovaleric and valeric acid concentrations were not significantly influenced by dietary treatment. The present study is in agreement with studies by Elliott et al. (1993) and Schauff et al. (1992) in which there were no effects of dietary treatment on concentrations of isovaleric and valeric acid in rumen fluid. The branch-chain VFA are isobutyrate and isovalerate acids. No significant differences were observed for the molar concentration of branch-chain fatty acids in the current study and few research studies indicate the concentration of these acids. Khorasani and Kennelly (1996) reported that as the inclusion level of heat treated canola seed increased from 3.75% to 14.5% (of dietary DM) the means of total branch-chain fatty acid concentrations increased linearly. In the study by

Jenkins and Jenny (1992), the concentration of branch-chain fatty acids increased in rumen fluid as fat content declined. Few studies indicate the molar concentration of caproate and as was found with the other VFA, caproate was not significantly different for the dietary treatments when 5% canola seed (dietary DM) was included in the diet. Khorasani et al. (1992) indicated that inclusion of heat treated canola seed at 4.5% (of dietary DM) also did not influence caproate concentrations in rumen fluid.

Khorasani et al. (1992) indicate that variability and differences in VFA production in research studies which utilize fat sources in the diet could be related to the level of fat inclusion, degree of saturation of fatty acids, forage to concentrate ratio, forage and/or grain source, diet and animal variation. It could be concluded that the inclusion level of 5% protected or unprotected canola seed (dietary DM), approximately 2.1% added dietary fat, did not significantly affect rumen fermentation characteristics.

3.4.7. Duodenal Nitrogen Fractions and Ruminal Bacterial Yield and Composition

No significant treatment effects were observed for N intake or duodenal N fractions suggesting that the rumen environment was not affected by adding 2.1% fat and rumen microorganisms were able to utilize the feedstuff as a N source. This would also suggest that CP digestibility, rumen bacteria and the rate or extent of protein degradation may not have been influenced by treatment. Crude protein digestion at the forestomach was, however, numerically lower with the formaldehyde treated canola seed diet in comparison to the other dietary treatments. This may suggest that the formaldehyde protection used may have reduced protein digestibility in the rumen but the effect was not large.

Hoover and Stokes (1991) reviewed the optimum conditions for rumen microbial yield. Factors influencing rumen microbial growth are chemical and physiological (ie: rumen pH, turnover rate) and nutritional (ie: feed intake, feeding strategies, forage length and quality and forage:grain ratio). Maximum rumen microbial protein per day is used as an index of rumen function. Limitations in sources and levels of N in the diet can result in depressed total carbohydrate digestion and, thus, lower microbial protein yield. It has been established that a reduced rumen pH decreases digestion of protein, cellulose and hemicellulose. It was

reported that a reduced rumen pH in the range of 6.5 to 5.5 decreases microbial efficiency (Mertens, 1979, Harfoot, 1978).

An increased output of microbial protein is desired to increase efficiency of animal production and reduce the protein requirement in feed. A variety of factors can affect microbial growth such as OM in the diet which influences anaerobic rumen fermentation. Anaerobic fermentation influences the amount of fermentation products (ie: acetate, propionate or butyrate) and energy (ATP). Microbial output is, therefore, the amount of OM digested in the ruminoreticulum and the efficiency with which the microbes use ATP from the degraded OM for growth (Ruckebusch et al., 1991). No significant differences were noted for OM intake or forestomach digestion for the dietary treatments and no statistical differences for bacterial composition and yield were found. DePeters et al. (1989) reports adding fat may reduce the energy available to the microbial population for growth which may alter microbial N utilization, however, in this experiment no significant differences were observed as a result of adding canola seed as a fat source.

Ruminal bacterial yield is the efficiency of microbial protein synthesis per unit of OM apparently digested in the rumen (ADOM). Murphy et al. (1987) found that microbial N synthesized in the rumen showed increased efficiency with increasing fat in the diet in terms of microbial N per kilogram of OM apparently digested in the rumen. The current study only utilized one level of fat and the addition of canola seed to the diet did result in a numerical increase in microbial efficiency when compared to the control, despite no significant differences. Ruckebusch et al. (1991) states microbial efficiency is often greater with less extensive digestion in the ruminoreticulum and with a lower total microbial yield. Rode et al. (1985) concluded that in their study the effect of diet on efficiency of microbial protein synthesis was primarily due to the physical nature of the diet rather than the chemical composition of the diet. Ørskov (1982) states that variations in the amount of microbial N produced are related to fermentation, rumen dilution rate and a host of other factors. In order to ensure maximum microbial protein is being produced the microbes require at least as much protein as the amount they incorporate into microbial biomass. It is assumed that the synchronization of N release required to achieve 100% efficiency of capture is impossible and

that in order to ensure that degradable N is not limiting microbial growth, an excess in required (Ørskov, 1982).

3.5. Conclusion

Dry mater intake, intakes of OM, CP, NDF, ADF, hemicellulose and cellulose, rumen fermentation characteristics and duodenal N fractions including bacterial composition and yield were not significantly altered by the dietary treatments utilized. Apparent forestomach digestibilities of DM, OM and CP were not influenced by the inclusion of canola seed (5% of dietary DM) in the diet, however, NDF, ADF, hemicellulose and cellulose were significantly influenced by the inclusion of canola seed (5% of dietary DM) diet. Whole tract digestibility of CP and hemicellulose was not influenced by dietary treatment. These results demonstrated that 5% added canola seed (approximately 2.1% added fat) to the diet as protected or unprotected seed did not influence the digestibility of nutrients in the rumen or in the whole digestive tract. The protection of canola seed with formaldehyde decreased the rate of protein degradation in the rumen. Changes in ruminal degradation characteristics of canola seed related to the heat treatment were rather small.

Figure 3.1. Diurnal patterns of ruminal pH as influenced by dietary treatment: control (◊), untreated canola seed (□), formaldehyde treated canola seed (▲) and heat treated canola seed (×).

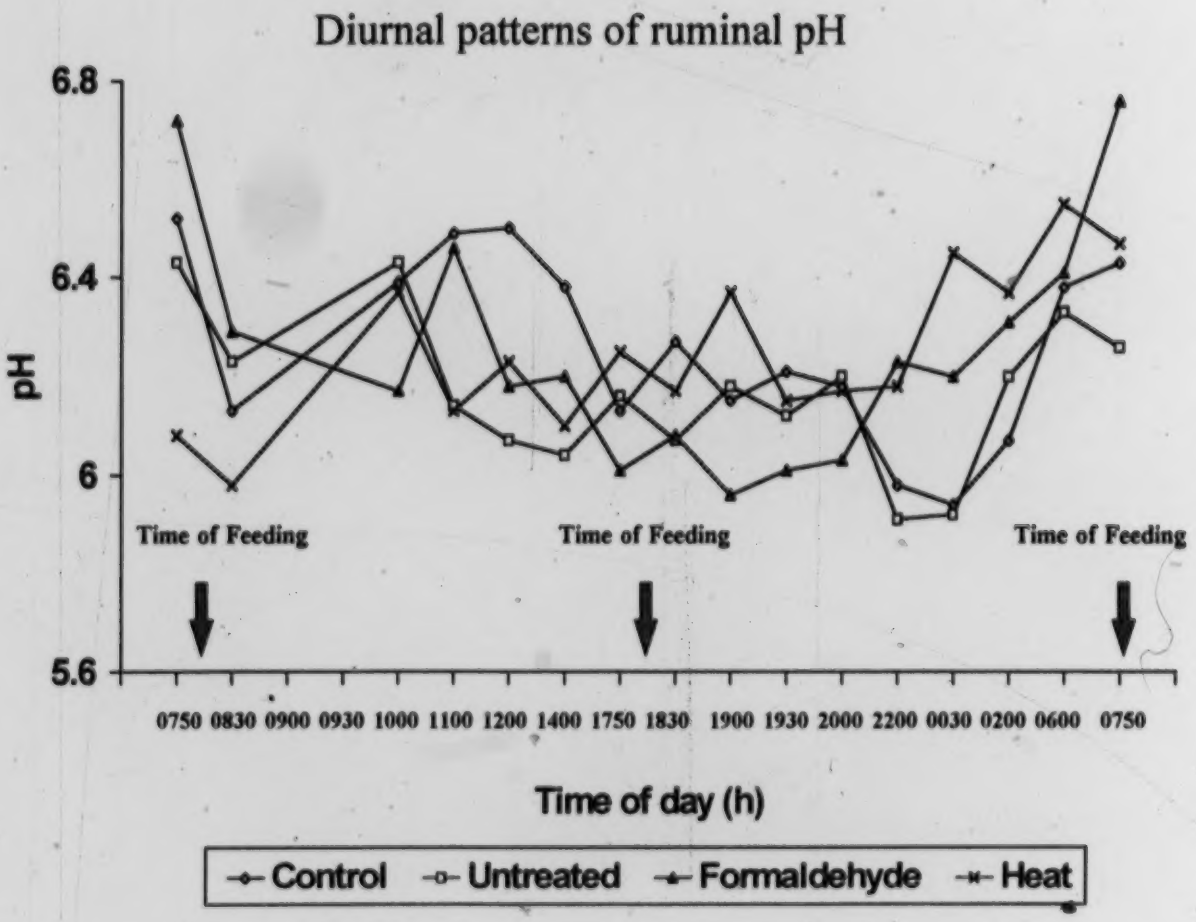

Diurnal patterns of ruminal pH
6.8
6.4
6
5.6
pH
Time of Feeding
Time of Feeding
Time of Feeding
0750 0830 0900 0930 1000 1100 1200 1400 1750 1830 1900 1930 2000 2200 0030 0200 0600 0750
Time of day (h)
Control
Untreated
Formaldehyde
Heat

Figure 3.2. Diurnal patterns of ruminal lactic acid as influenced by dietary treatment: control (◊), untreated canola seed (□), formaldehyde treated canola seed (▲) and heat treated canola seed (×).

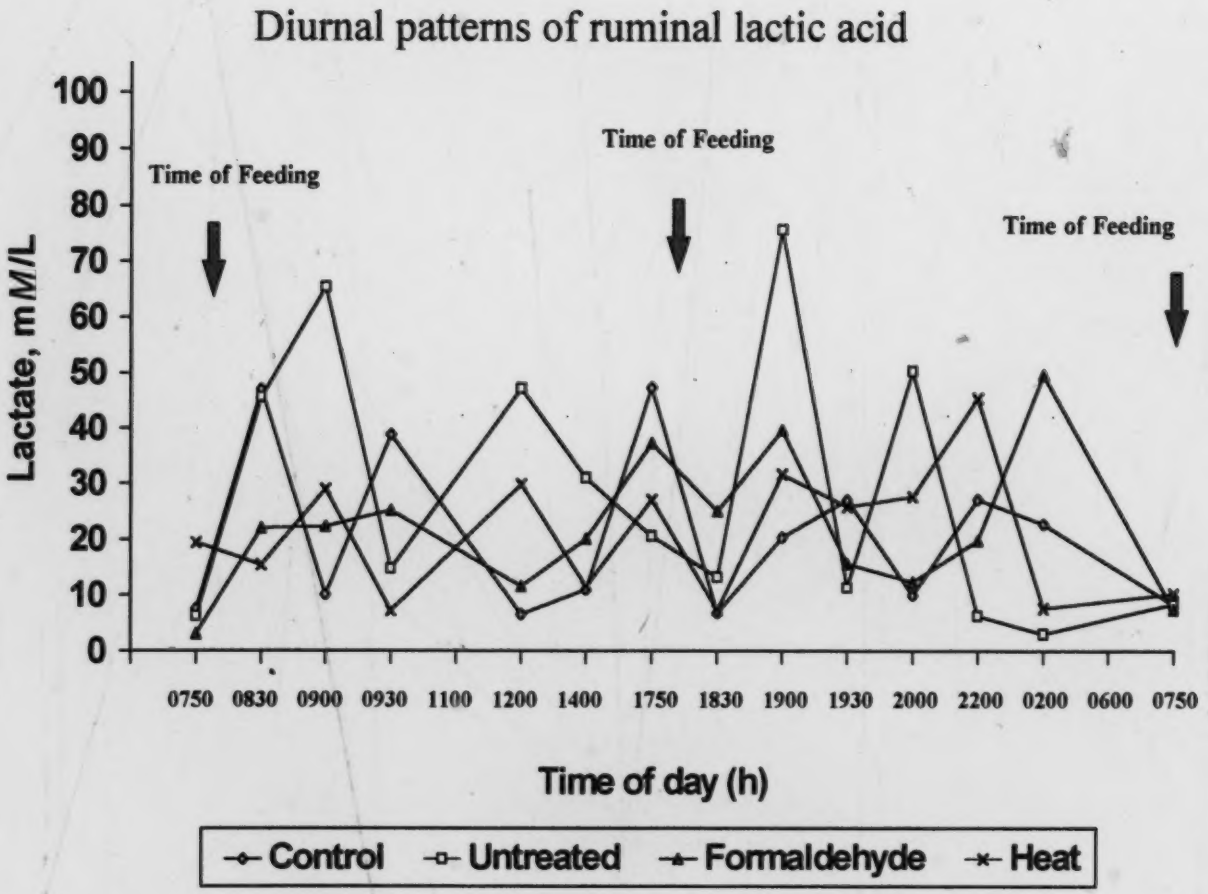

Diurnal patterns of ruminal lactic acid
Lactate, mM/L
100
90
80
70
60
50
40
30
20
10
0
Time of Feeding
Time of Feeding
Time of Feeding
0750 0830 0900 0930 1100 1200 1400 1750 1830 1900 1930 2000 2200 0200 0600 0750
Time of day (h)
Control
Untreated
Formaldehyde
Heat

Figure 3.3. Diurnal patterns of ruminal ammonia nitrogen as influenced by dietary treatment: control (◊), untreated canola seed (□), formaldehyde treated canola seed (▲) and heat treated canola seed (×).

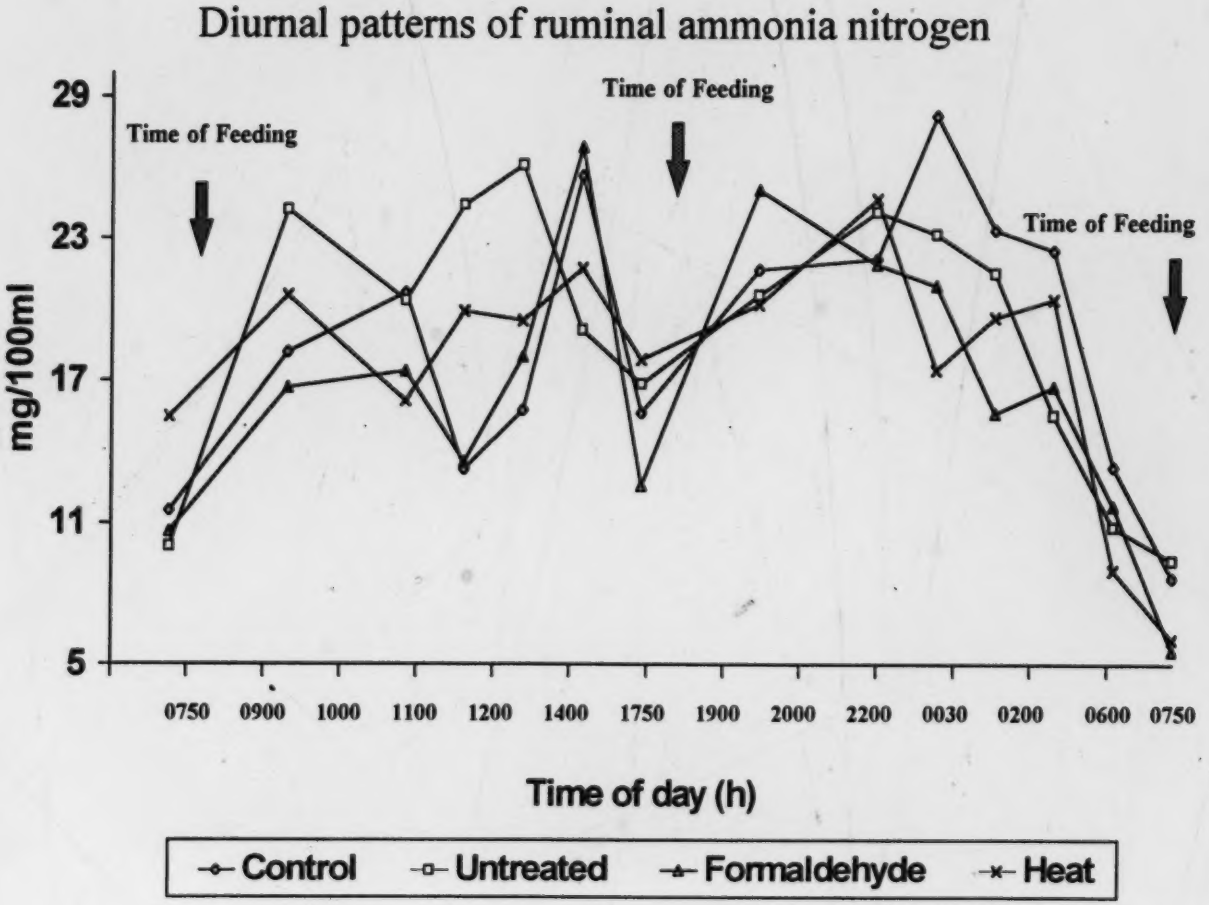

Diurnal patterns of ruminal ammonia nitrogen
Time of Feeding
Time of Feeding
Time of Feeding
29
23
17
11
5
mg/100ml
0750
0900
1000
1100
1200
1400
1750
1900
2000
2200
0030
0200
0600
0750
Time of day (h)
Control
Untreated
Formaldehyde
Heat

Figure 3.4. Diurnal patterns of total volatile fatty acids in rumen fluid as influenced by dietary treatment: control (◊), untreated canola seed (□), formaldehyde treated canola seed (▲) and heat treated canola seed (×).

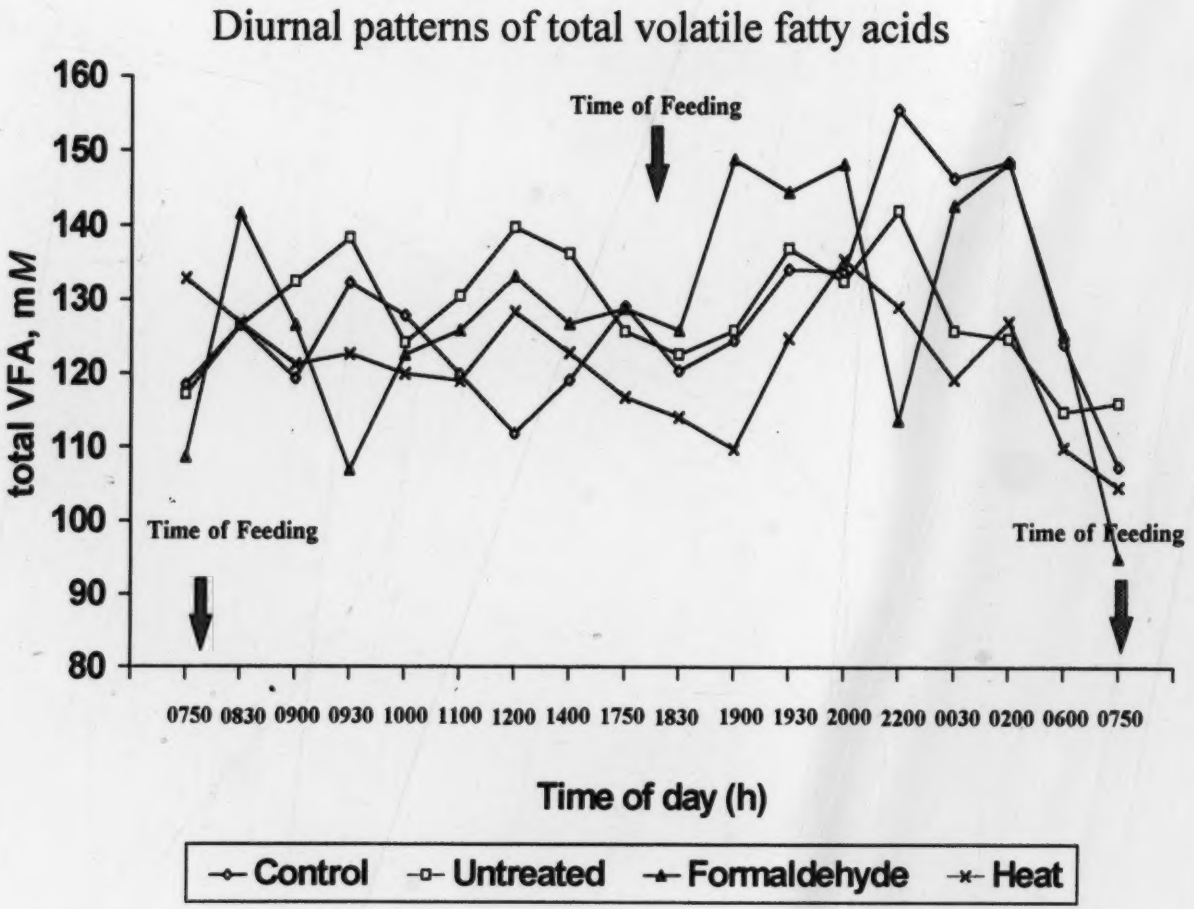

Diurnal patterns of total volatile fatty acids
160
150
140
130
120
110
100
90
80
total VFA, mM
Time of Feeding
Time of Feeding
Time of Feeding
0750 0830 0900 0930 1000 1100 1200 1400 1750 1830 1900 1930 2000 2200 0030 0200 0600 0750
Time of day (h)
Control
Untreated
Formaldehyde
Heat

Figure 3.5. Diurnal patterns of acetate in rumen fluid as influenced by dietary treatment: control (◊), untreated canola seed (□), formaldehyde treated canola seed (▲) and heat treated canola seed (×).

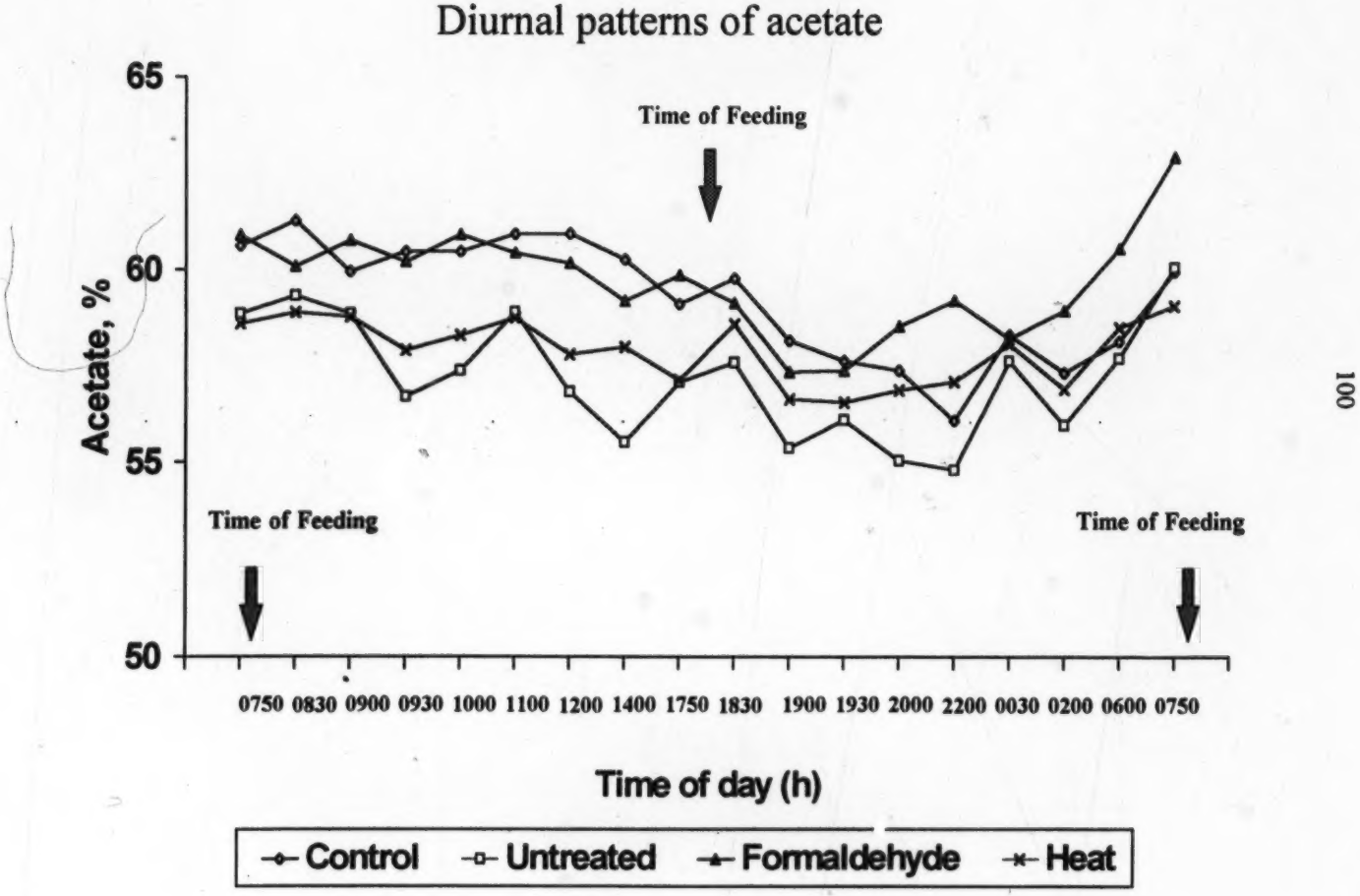

Diurnal patterns of acetate
65
60
55
50
Acetate, %
Time of Feeding
Time of Feeding
Time of Feeding
0750 0830 0900 0930 1000 1100 1200 1400 1750 1830 1900 1930 2000 2200 0030 0200 0600 0750
Time of day (h)
Control
Untreated
Formaldehyde
Heat

Figure 3.6. Diurnal patterns of propionate in rumen fluid as influenced by dietary treatment: control (◊), untreated canola seed (□), formaldehyde treated canola seed (▲) and heat treated canola seed (×).

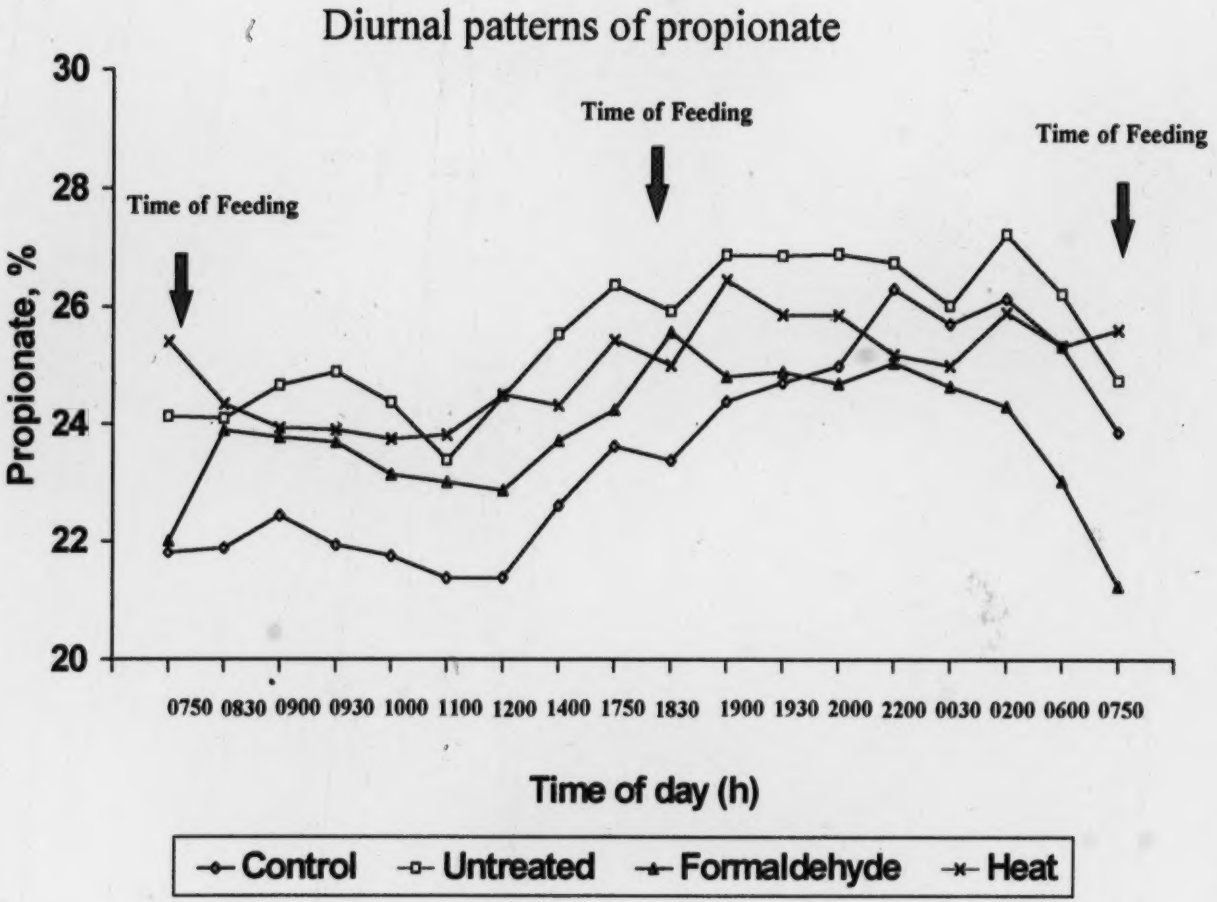

Diurnal patterns of propionate
Time of Feeding
Time of Feeding
Time of Feeding
Propionate, %
30
28
26
24
22
20
0750 0830 0900 0930 1000 1100 1200 1400 1750 1830 1900 1930 2000 2200 0030 0200 0600 0750
Time of day (h)
Control
Untreated
Formaldehyde
Heat

Figure 3.7. Diurnal patterns of isobutyrate in rumen fluid as influenced by dietary treatment: control (◊), untreated canola seed (□), formaldehyde treated canola seed (▲) and heat treated canola seed (×).

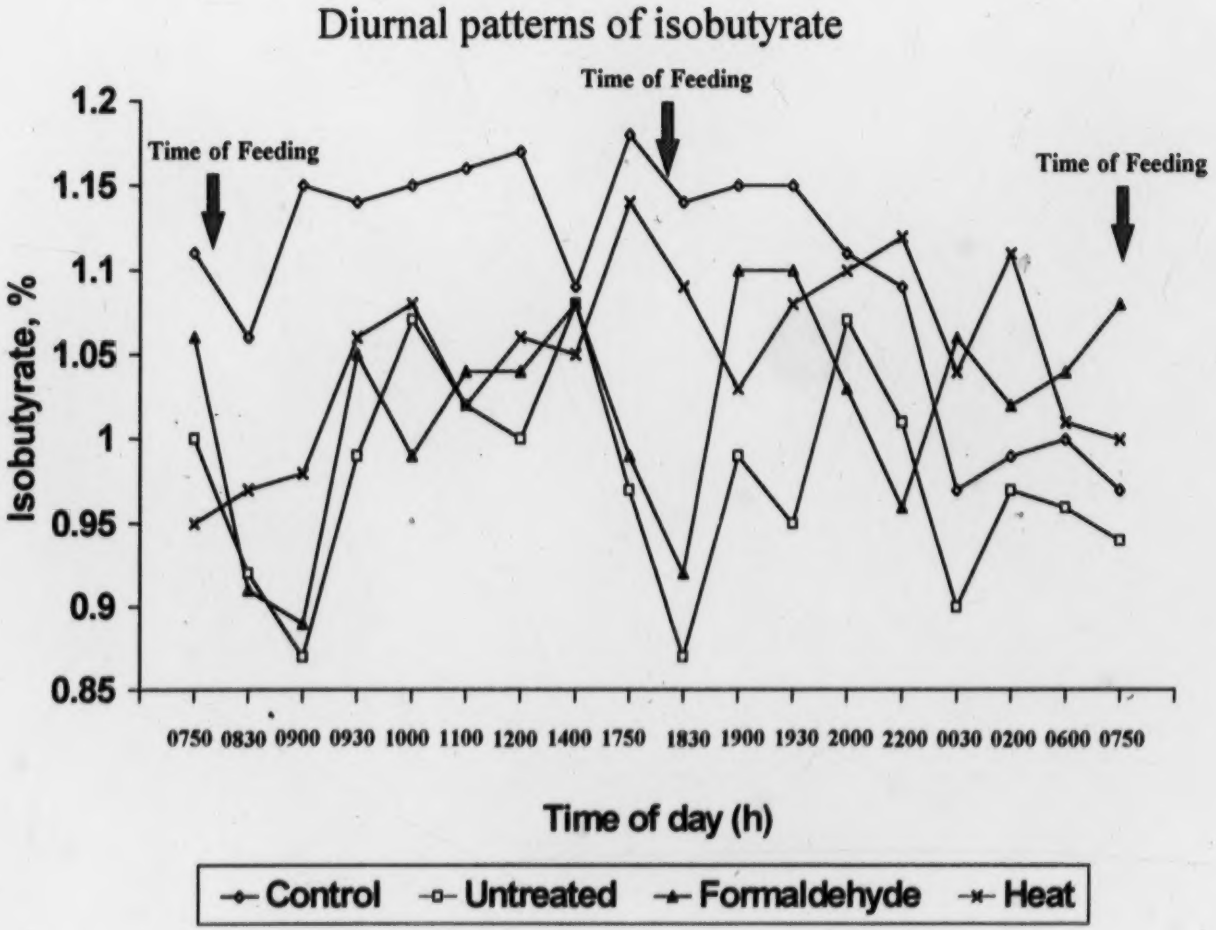

Diurnal patterns of isobutyrate
Time of Feeding
Time of Feeding
Time of Feeding
Isobutyrate, %
1.2
1.15
1.1
1.05
1
0.95
0.9
0.85
0750 0830 0900 0930 1000 1100 1200 1400 1750 1830 1900 1930 2000 2200 0030 0200 0600 0750
Time of day (h)
Control
Untreated
Formaldehyde
Heat

Figure 3.8. Diurnal patterns of butyrate in rumen fluid as influenced by dietary treatment: control (◊), untreated canola seed (□), formaldehyde treated canola seed (▲) and heat treated canola seed (×).

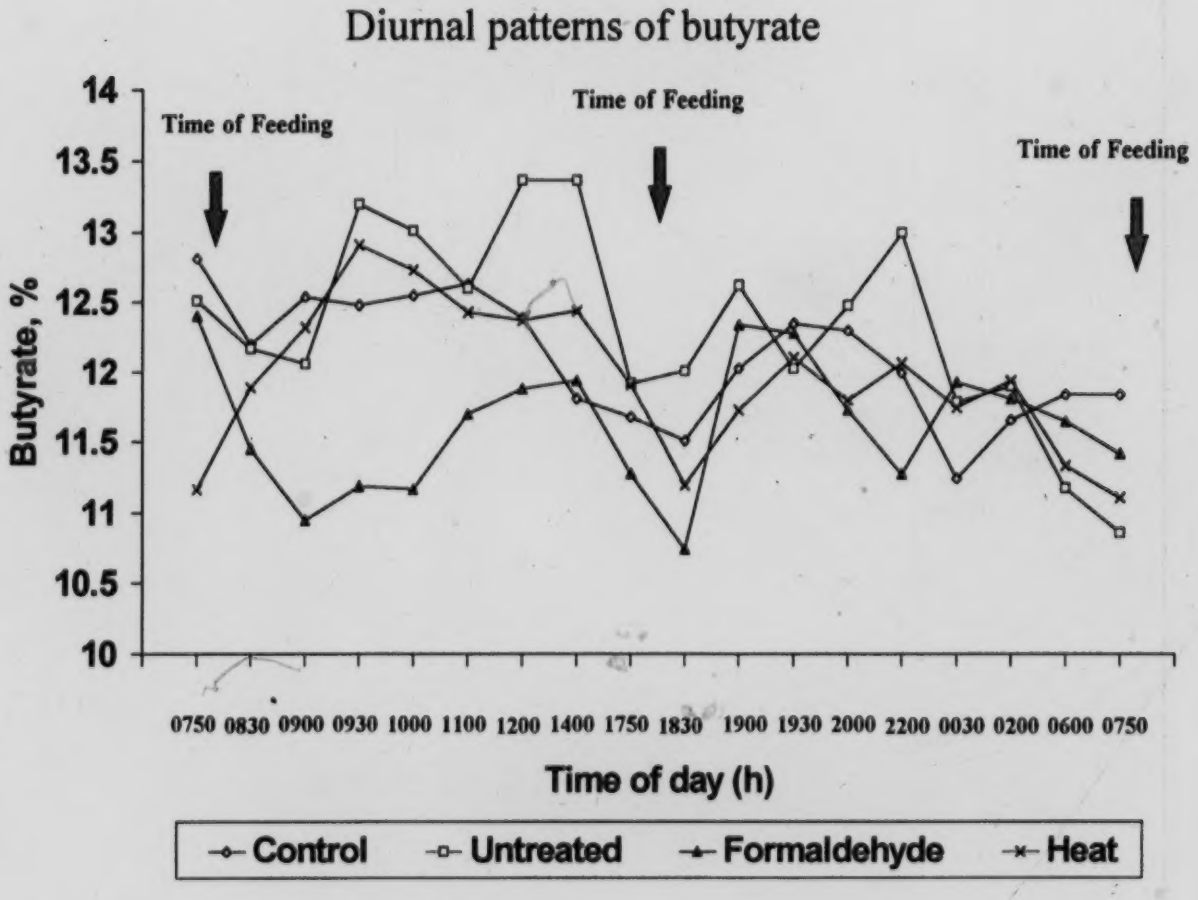

Diurnal patterns of butyrate
Time of Feeding
Time of Feeding
Time of Feeding
Butyrate, %
14
13.5
13
12.5
12
11.5
11
10.5
10
0750 0830 0900 0930 1000 1100 1200 1400 1750 1830 1900 1930 2000 2200 0030 0200 0600 0750
Time of day (h)
Control
Untreated
Formaldehyde
Heat

Figure 3.9. Diurnal patterns of isovalerate in rumen fluid as influenced by dietary treatment: control (◊), untreated canola seed (□), formaldehyde treated canola seed (▲) and heat treated canola seed (×).

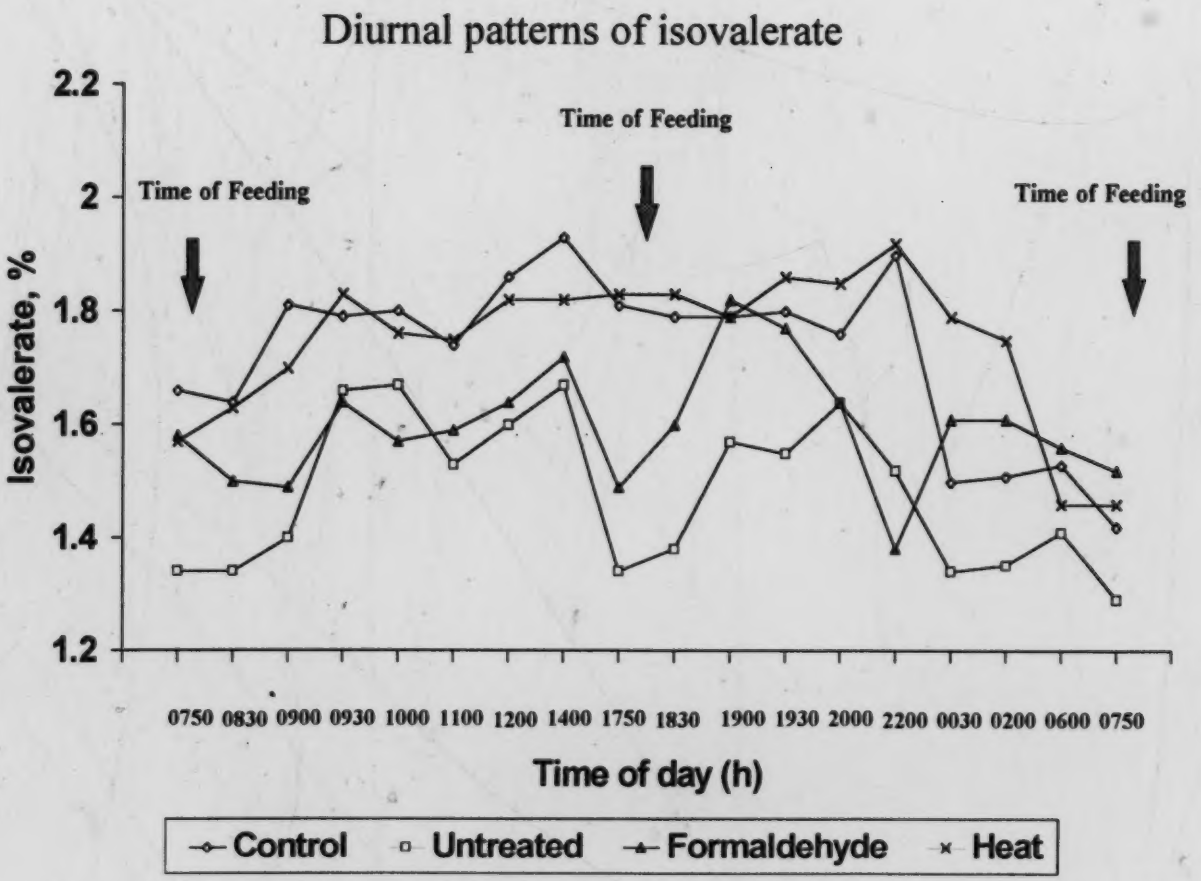

Diurnal patterns of isovalerate
Time of Feeding
Time of Feeding
Time of Feeding
Isovalerate, %
2.2
2
1.8
1.6
1.4
1.2
0750 0830 0900 0930 1000 1100 1200 1400 1750 1830 1900 1930 2000 2200 0030 0200 0600 0750
Time of day (h)
Control
Untreated
Formaldehyde
Heat

Figure 3.10. Diurnal patterns of valerate in rumen fluid as influenced by dietary treatment: control (◊), untreated canola seed (□), formaldehyde treated canola seed (▲) and heat treated canola seed (×).

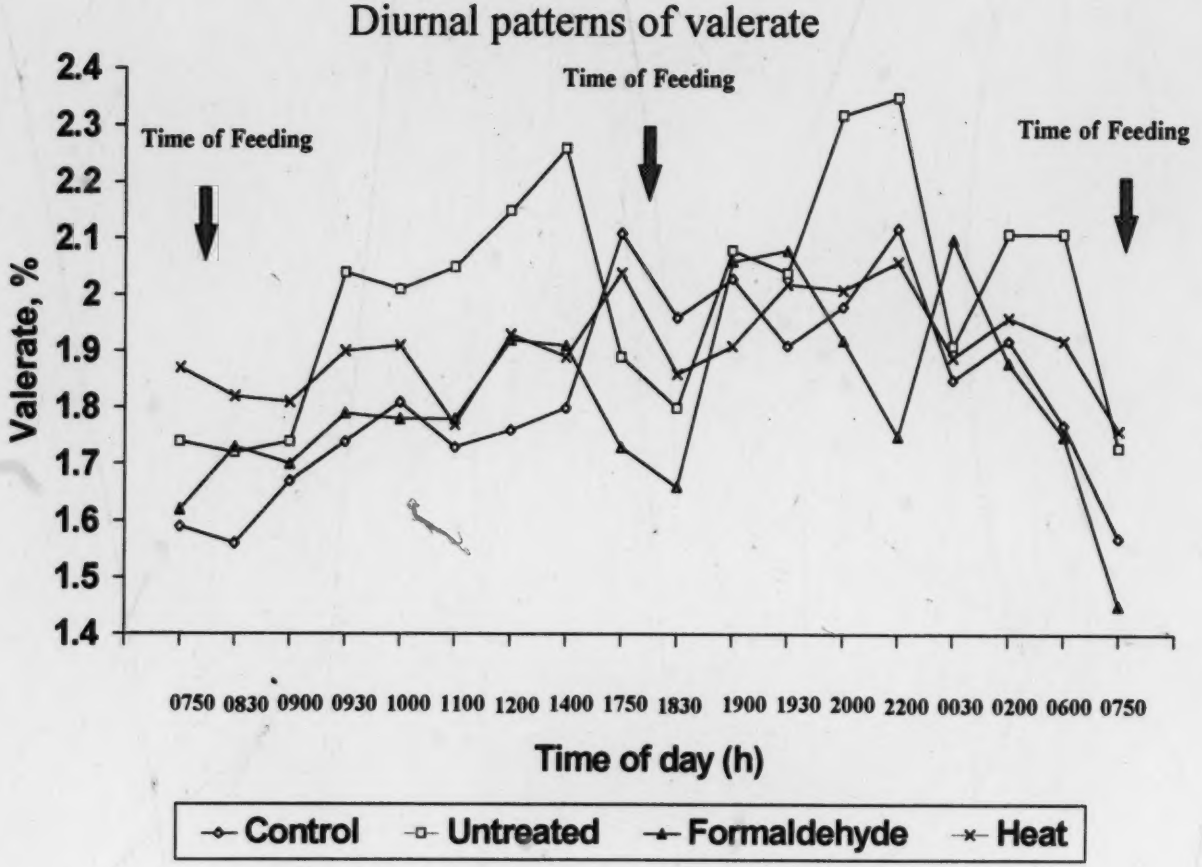

Diurnal patterns of valerate
Time of Feeding
Time of Feeding
Time of Feeding
Valerate, %
2.4
2.3
2.2
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
0750 0830 0900 0930 1000 1100 1200 1400 1750 1830 1900 1930 2000 2200 0030 0200 0600 0750
Time of day (h)
Control
Untreated
Formaldehyde
Heat

Figure 3.11. Diurnal patterns of branched chain volatile fatty acids in rumen fluid as influenced by dietary treatment: control (◇), untreated canola seed (□), formaldehyde treated canola seed (▲) and heat treated canola seed (×).

Diurnal patterns of isobutyrate and isovalerate (branch chain)

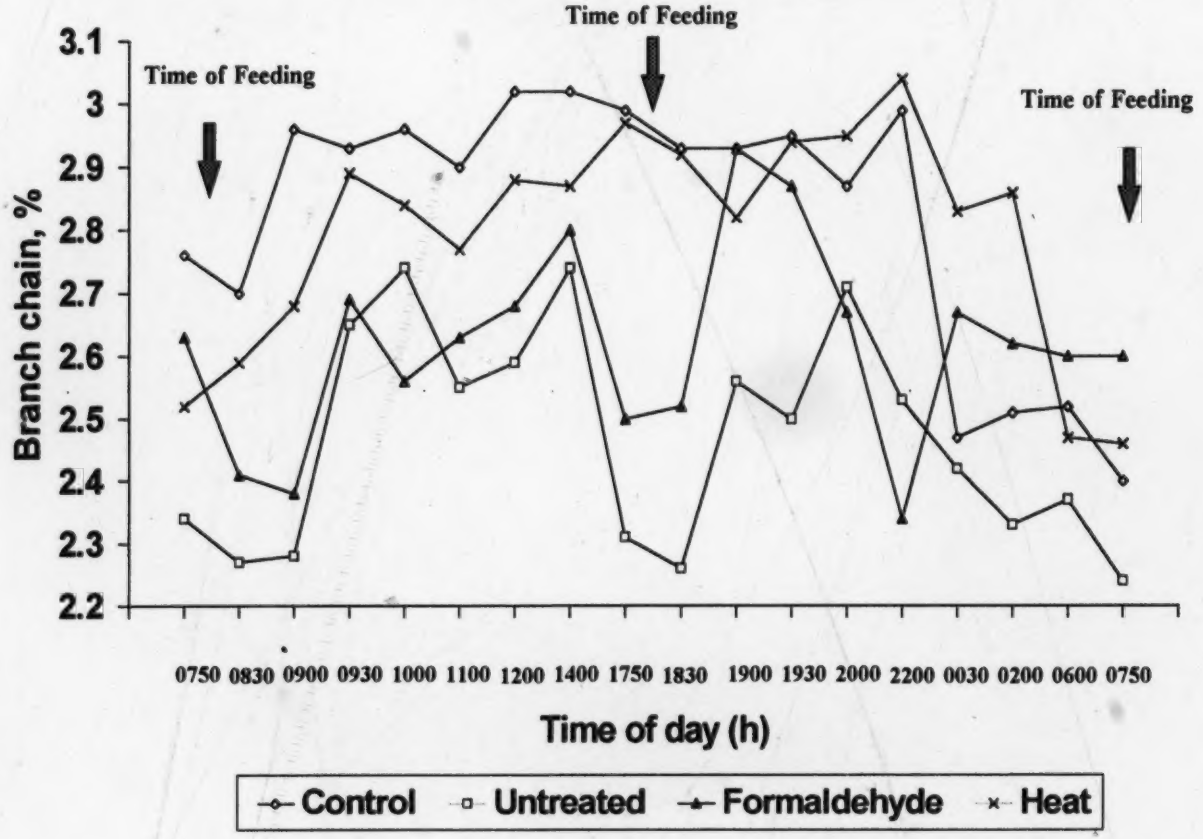


Figure 3.12. Diurnal patterns of caproate in rumen fluid as influenced by dietary treatment: control (◊), untreated canola seed (□), formaldehyde treated canola seed (▲) and heat treated canola seed (×).

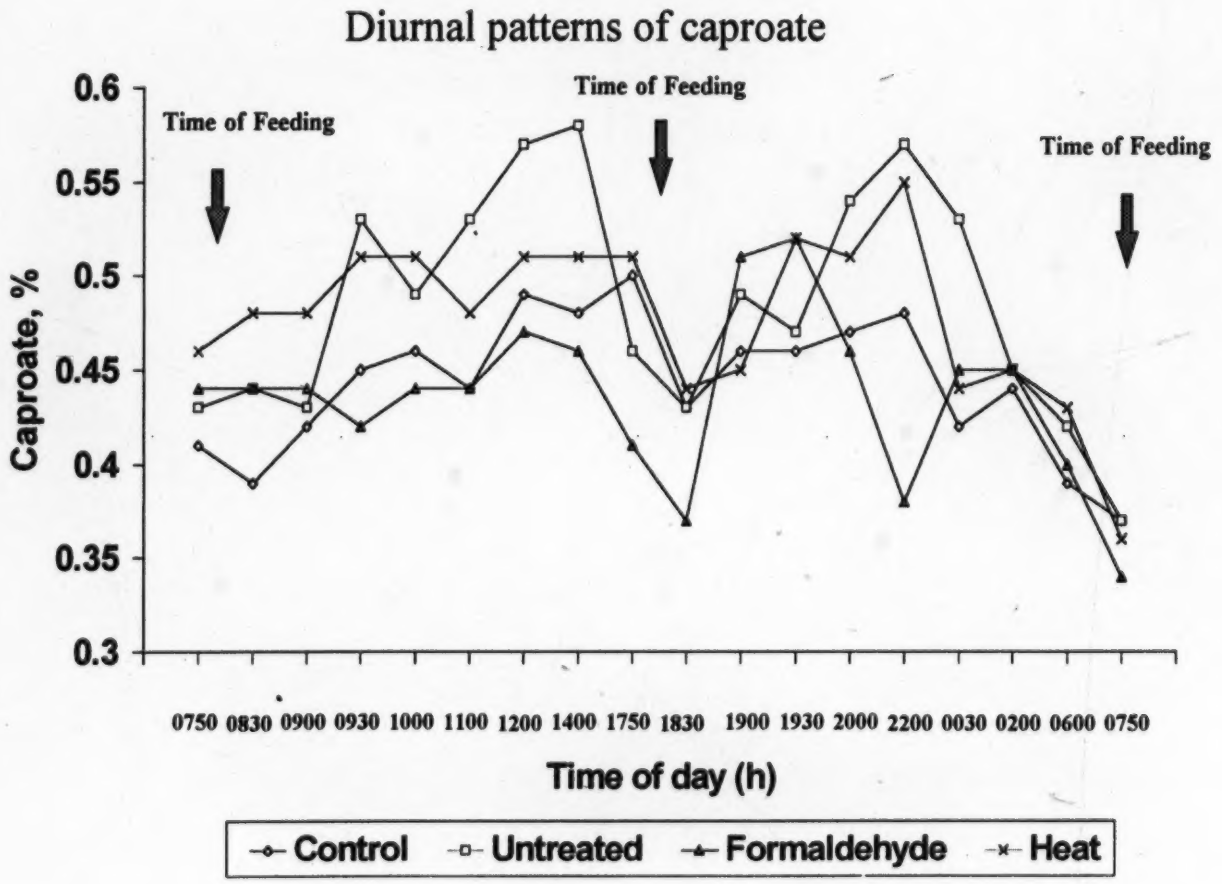
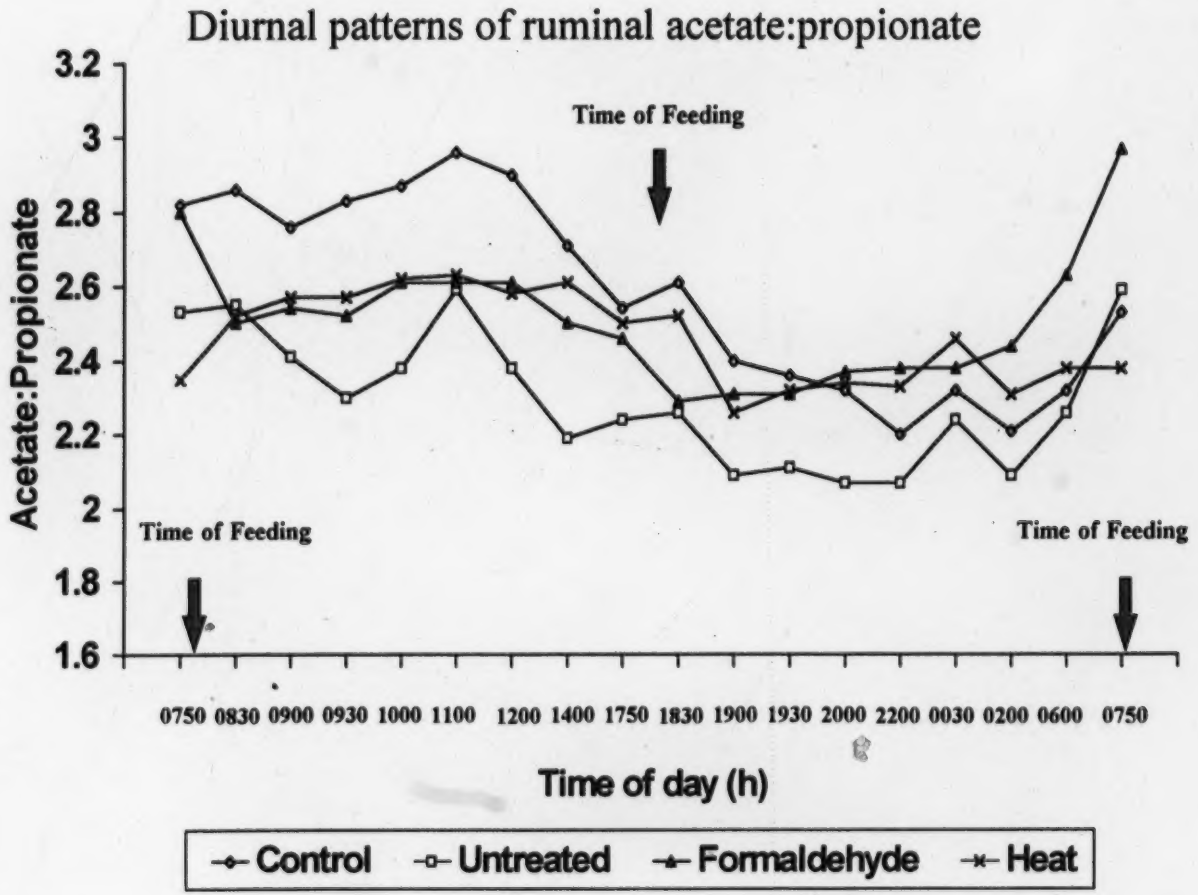

Diurnal patterns of caproate
Time of Feeding
Time of Feeding
Time of Feeding
Caproate, %
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0750 0830 0900 0930 1000 1100 1200 1400 1750 1830 1900 1930 2000 2200 0030 0200 0600 0750
Time of day (h)
Control
Untreated
Formaldehyde
Heat

Figure 3.13. Diurnal patterns of acetate:propionate acids in rumen fluid as influenced by dietary treatment: control (◊), untreated canola seed (□), formaldehyde treated canola seed (▲) and heat treated canola seed (×).


Diurnal patterns of ruminal acetate:propionate
Acetate:Propionate
3.2
3
2.8
2.6
2.4
2.2
2
1.8
1.6
Time of Feeding
Time of Feeding
Time of Feeding
0750 0830 0900 0930 1000 1100 1200 1400 1750 1830 1900 1930 2000 2200 0030 0200 0600 0750
Time of day (h)
Control
Untreated
Formaldehyde
Heat

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CHAPTER 4

Effect of Dietary Fat on Milk Yield and Milk Composition in Holstein Cows.

4.1. Introduction

Over the past few decades, milk production and feeding techniques for dairy cattle have changed. Researchers have devoted time to projects to improve lactating dairy cow diets in order for the cow to achieve her genetic potential for milk production. Increasing the amount of concentrate in the diet and improving forage quality have been the major foci of dairy nutrition research. During early-lactation, dairy cows are in a negative energy balance and rely on the mobilization of body reserves to meet their energy requirement. High concentrate diets have been utilized to overcome the substantial energy deficit in early-lactation, however, these high concentrate diets tend to depress milk fat percentage (Khorasani et al., 1991). Energy requirements for lactation can be more effectively met by feeding fat which has a higher concentration of energy compared to carbohydrates and proteins, approximately 2.25 times greater (Palmquist and Jenkins, 1980, Miller, 1979).

Including high concentrations of fat into the lactating dairy diet, however, can adversely effect rumen fermentation and nutrient digestibility (Palmquist and Jenkins, 1980). In order to overcome the detrimental effects of fat on rumen function, the fat source must be protected from rumen microbial degradation. A major technological breakthrough in the area of dietary fat usage by ruminants is "protecting" the fat supplement. A number of researchers have researched and reviewed the area of adding dietary fat, such as canola seed, to the dairy cow ration in order to alter fatty acid composition of milk fat (Ashes et al., 1992, Chilliard, 1993, Grummer, 1991). Methods of protection which have been utilized to protect fat sources from rumen degradation include heat (ie: Jet-Sploding® or extrusion) (Khorasani et al., 1991, Deacon et al., 1988) and chemical treatment (ie: formaldehyde) (Ashes et al., 1995, 1992, Atwal et al., 1991). A protected or modified fat supplement is generally referred to as rumen inert or rumen bypass fat and is able to escape rumen biohydrogenation and hydrolysis resulting in the polyunsaturated fatty acids being digested and absorbed postruminally and,

thus, being incorporated into milk fat. Other forms of feeding dietary fat to minimize the detrimental effects on ruminal fermentation include addition of calcium salts, prilled, or partially hydrogenated fat sources (Drackley and Elliott, 1993, Jenkins and Jenny, 1989, Grummer, 1988).

The reason for canola seed being used as a protected fat source is that canola seed contains approximately 40% lipid of which 90% is in the form of unsaturated fatty acids. The principle fatty acids in canola include oleic ($C_{18:1}$, 60%), linoleic ($C_{18:2}$, 30%) and linolenic ($C_{18:3}$, 10%) (Kennelly, 1996a,b, Ashes et al., 1992, Atwal et al., 1991). Canola seed is also an attractive oilseed to dairy producers in Western Canada due to its relatively high protein content (22% CP) (Rode, 1989). Due to the high fat content of canola seed, the level in which canola seed can be added to the diet must be limited in order to prevent any deleterious effects on microbial digestion in the rumen. Protection methods used to prevent the deleterious effects of fat in the rumen, therefore, have received much attention. The potential to use canola seed as a lipid to alter the fatty acid composition of milk is also an area of focus.

Despite milk being one of nature's most perfect foods because of its balanced availability of protein, fat, carbohydrates, vitamins and minerals, there is pressure from the medical profession to decrease the amount of saturated fats and total amount of fat in the consumer's diet due to linking saturated fat to cardiovascular diseases. The consumer trend is to consume low fat products and less saturated fat. For example, consumers are substituting 2%, 1% or skim milk for whole milk or not consuming dairy products such as butter. Interest in using protected lipid sources in the diet of lactating dairy cows, therefore, has been generated from the consumer demand for less saturated fat in dairy products and the decline in consumption of milk fat.

The hypothesis of the study was that heat or chemical treatment of canola seed would increase the supply of unsaturated fatty acids to the intestine and, thus, provides an efficacious method of altering the fatty acid composition of milk. The objective of this study, therefore, was to examine the effects of heat and chemical (formaldehyde) treatment of canola seed on fatty acid composition of milk fat.

4.2. Materials and Methods

4.2.1. Animals and Experimental Design

Four multiparous, ruminally and duodenally cannulated Holstein dairy cows in early-lactation (82 $\pm$ 20 DIM) were assigned to four treatments. The Holstein cows were fitted with a large rumen cannula (#1C-rumen cannula with rolled inner flange, 10 cm centre diameter, Bar Diamond Inc. Parma, ID) and a hard cannula (T-type) at the proximal duodenum. The care of the dairy cows was in accordance with the Animal Use for Research or Teaching Protocol approved by the Animal Use Committee at the University of Alberta. Cows were randomly assigned to one of four treatments in a 4 x 4 Latin square design. An additional ruminally cannulated cow (82 DIM) in early-lactation was available and used for generating additional data for milk yield and milk composition. This additional cow was assigned throughout the whole study with the same cow.

Each experimental period was three weeks in length which consisted of a change-over period for the first three days, and adaptation period from day 4 to 14, and a collection period from day 15 to 21. The change-over period involved increasing the new diet each day by 25% until day four when the new diets were fed at 100%. Cows were tethered in tie stalls and had free access to water.

4.2.2. Diets

A control diet was formulated to satisfy the nutrient requirements of a Holstein cow, weighing 600 kg, producing 40 kg milk per day of average composition (30 g of butterfat per kg milk), according to the NRC (1989). The control diet consisted of 60% concentrate and 40% forage (alfalfa silage and barley silage as a 50:50 ratio) on a dry matter (DM) basis. The test concentrates supplied 1 kg/day (5% of diet on DM basis) of untreated, formaldehyde treated and heat treated canola seed. Canola seed replaced canola meal and barley in the control concentrate. Fat content of the canola seed was assumed to be 40% as indicated by research of Ashes et al. (1992). The concentrate mixtures were (1) control with no added fat (Control), (2) control + unprotected rolled canola seed, (3) control + formaldehyde protected canola seed and (4) control + heat treated canola seed. A total mixed ration (TMR) was fed

ad libitum to all cows at 0800 h and 1800 h. The total mixed ration was fed twice daily with two-thirds at 0800 h and one-third at 1800 h. Dry matter intake was recorded daily and adjusted to maintain 5 to 10% orts. Refusal amounts were recorded prior to each morning feeding. Table 3.2 illustrates the composition of the concentrates on an as fed basis and Table 3.3 the composition of the TMR on a DM basis.

4.2.3. Canola Seed Treatments

The formaldehyde treated canola seed was acquired from Dr. John Ashes of the Commonwealth Scientific Industrial Research Organization (CSIRO), Blacktown, New South Wales, Australia. The procedure for formaldehyde treatment of oilseeds involves computer aided control systems which mix the oilseed (dehulled) together with an antioxidant. The seed is then homogenized with water and alkali is introduced to solubilize the protein as outlined by the procedures of Scott and Ashes (1993) and Ashes et al. (1992). The formaldehyde treated canola seed consisted of 70% canola seed, 30% full fat soybeans and ethoxyquin (antioxidant) at 600 ppm. The untreated canola seed and the heat treated canola seed were both acquired from Mary Lou Swift of ProFORM Feeds Inc., Chilliwack, British Columbia, Canada. The untreated canola seed was rolled prior to feeding in the production study. The heat treated canola seed was heated at 120°C for 15 min. No moisture was added during the heating process.

4.2.4. Body Condition Score and Live Weight

Body condition score (BCS), to the nearest 0.25 unit, and body weight were recorded on the first and second day of each experimental period.

4.2.5. Milk Yield and Milk Composition

Milk yield was electronically recorded daily for the duration of the study. Milk samples, collected from Monday p.m. and Tuesday a.m. milking each week, were analyzed for fat, protein and lactose by infrared analysis at the Alberta Central Milk Testing Laboratory (Edmonton, Alberta, Canada). Milk samples collected from Monday p.m. (day 17) and

Tuesday a.m. (day 18) milking during the collection week were analyzed for fat, fatty acid composition, crude protein (total N), true protein (protein N), casein nitrogen (casein N), casein protein (kappa, alpha and beta proteins), whey protein (lactoglobulin and lactalbumin), non-protein nitrogen (NPN) and free fatty acids (FFA) at the University of Alberta laboratory. Minerals in the milk samples were analyzed at the Alberta Central Milk Testing Laboratory (Edmonton, Alberta, Canada). The milk samples which were collected during the collection week were pooled according to production (day 17 and 18). All milk sample analysis was done in duplicate.

4.2.6. Chemical Analysis of Milk

Fatty acids were extracted using 2:1 chloroform:methanol (v/v) (Jensen et al., 1985), drying under N and methylating with boron triflouride/methanol (AOAC, 1984). After esterification (Morrison and Smith, 1964) the fatty acid profiles of the individual samples were measured by gas chromatography. Conditions of the gas chromatograph are stated in Appendix 4.1. The peak areas for each fatty acid were processed with Shimadzu Ezchrom 2.1 version data system (Shimadzu Scientific Instruments, Inc., 7102 Riverwood Drive, Columbia MD, 21046) and expressed as percentages of total fatty acids detected. Identification of the fatty acid peaks was based on the retention times of standard methyl esters of individual fatty acids. The procedure as described by Lien and Kennelly (1996) and modified from de Jong and Badings (1990) and the International Dairy Federation (1991) was utilized to determine the FFA content of milk. Free fatty acids were determined by pipetting 10 ml of milk and extracting the FFA in 15 ml of cold ether:heptane (1:1v/v) and isolating on an anion exchange column (Supelco Ltd., Mississauga, Ontario, Canada). The procedure is outlined in Appendix 4.2. Determination of the amount of FFA in milk was based upon the amount of internal standard added to the sample.

Total milk N was determined by the Kjeldahl procedure (AOAC, 1984). Casein N was determined by precipitating casein (in 2.5 ml milk), using a mixture of acetic acid (0.5 ml, 10%) and sodium acetate (0.4 ml, 1M), filtering, and analyzing the filtrate for N using the Kjeldahl procedure (AOAC, 1984). True protein N was determined by precipitating total

protein (in 5 ml milk) using 24% TCA, adding 10 ml of 12% TCA during the filtering process as a rinse and analyzing the filtrate for N using the Kjeldahl procedure (AOAC, 1984). Non-protein N was determined as the difference between total N and true protein N. Whey protein N was determined as the difference between true protein N and casein N (Gordon and Kalan, 1983).

The procedure as described by Lien et al. (1996a,b) and modified from Visser et al.. (1991) and Poll and Harding (1991) was used to determine the content of casein and whey proteins in milk, respectively. Casein protein was determined by precipitating casein (in 2.5 ml milk) using a mixture of water (2 ml), acetic acid (10%) and sodium acetate (0.1N). The procedure is outlined in Appendix 4.3. For determination of casein proteins in milk, a standard curve was calculated from the amount of individual casein proteins in the standard and their peak area responses. The amount of individual casein in each milk sample was calculated by extrapolation of its peak area response to amounts using the standard curve. To isolate whey proteins, the casein procedure (Lien et al., 1996b) was used up to the casein isolation step in which the aqueous layer was transferred to a chromatography vial. The procedure is outlined in Appendix 4.4. The calculation to determine the content of whey proteins in milk involved a standard curve being calculated from the amount of individual whey proteins in the standard and their peak area response. The amount of individual whey proteins in each milk sample was calculated by extrapolation of their peak responses to the amounts using the standard curve.

Minerals in milk were determined by freeze drying 15 ml of milk at -60°C with no shelf heat for 24 h, after which the shelf heat (15°C) was turned on and samples were dried to complete dryness. Mineral analysis was done using the inductively coupled plasma (ICP) emission spectrometer according to the procedures at the Alberta Central Milk Testing Laboratory (Edmonton, Alberta, Canada). The minerals studied were iron (Fe), magnesium (Mg), aluminum (Al), sulphur (S), copper (Cu), potassium (K), zinc (Zn), phosphorous (P), manganese (Mn), sodium (Na), boron (B) and calcium (Ca).

4.2.7. Statistical Analysis

The mean of DM intake, milk yield, milk composition and milk component production for the last seven days of each period were used to compare the effect of each treatment. The data was analyzed as Latin square analysis using a model which included cow, period and diet. The procedure of general linear models (GLM) in SAS (1995) was used to analyze the data for milk yield and composition, body weight and BCS. The data least square means (lsmeans) were compared at a significance level of $P<0.05$ using pdiff and the lsmeans were used to illustrate the data.

4.3. Results

4.3.1. Cow Performance

Body weight (kg) for cows fed the four dietary treatments was not significantly different (Table 4.1). Daily milk, fat, protein and lactose yield (kg/day) were not influenced by dietary treatment (Table 4.1). The dietary treatments, however, did affect milk fat percentage but milk protein and lactose percentages were not altered by dietary treatment. Milk fat percentage was lower for cows fed the untreated canola seed compared to the other dietary treatments, however, no significant differences were observed among the other dietary treatments.

4.3.2. Milk Fatty Acid Composition

Milk fatty acid composition was altered by the canola seed treatments (Tables 4.2, 4.3, 4.4, 4.5). Caprylic acid ($C_{8:0}$) concentration (Table 4.2) for milk from cows fed the formaldehyde treated and heat treated canola seed diets was significantly different. Caprylic acid in milk from cows fed the control and untreated canola seed diets were similar to the formaldehyde treated and heat treated canola seed milk. Cows fed the formaldehyde treated canola seed diet had significantly lower $C_{12:1}$ in milk in comparison to the cows fed the control and untreated canola seed diets.

Myristic acid ($C_{14:0}$) was not influenced by dietary treatment (Table 4.2), however, $C_{14:0}$ in milk for cows fed the control and untreated canola seed tended to be higher than in milk from cows fed formaldehyde treated ($P = 0.07$) and heat treated canola seed ($P = 0.09$).

Table 4.1. Least square means for milk yield and milk composition for cows fed the dietary treatments.

Item	Diet				SEM
	Control	Untreated	Formaldehyde treated	Heat treated	
DM intake (kg/day)	20.3	22.8	20.7	20.3	0.97
Body Weight (kg)	685.9	704.0	679.3	685.6	14.77
BCS	2.64	2.81	2.73	2.65	0.16
Yield (kg/day)					
Milk	31.9	35.5	33.3	32.5	1.57
Fat	1.28	1.14	1.30	1.24	0.07
Protein	1.02	1.13	1.04	1.03	0.05
Lactose	1.48	1.66	1.51	1.50	0.08
Composition, %					
Fat	3.93[a]	3.18[b]	3.82[a]	3.71[a]	0.07
Protein	3.13	3.15	3.05	3.11	0.07
Lactose	4.58	4.66	4.54	4.57	0.04

[a, b] Means in the same row with different superscripts differ ($P<0.05$)

Inclusion of canola seed in the diet resulted in a decline in the concentration of $C_{16:0}$ (total) with the greatest decline observed for cows fed the untreated and formaldehyde treated canola seed treatments. The concentration of $C_{16:1}$ in milk for cows fed the formaldehyde treated canola seed was lower than from cows fed the control and heat treated canola seed diets. With one exception, the concentration of $C_{17:0}$ and $C_{17:1}$ in milk (Table 4.3) declined with the inclusion of the canola seed in the diet. The concentration of these two fatty acids was less than 1.2% of the total fatty acids found in milk.

The concentration of stearic acid ($C_{18:0}$) increased with the inclusion of canola seed in the diet compared to the control (Table 4.4). Cows fed the formaldehyde treated and heat treated canola seed diets had similar concentrations of stearic acid but were lower than cows

fed the untreated canola seed diet. Oleic acid ($C_{18:1}$) was identified as either *cis* or *trans* and

Table 4.2. Least square means for short- and medium-chain fatty acids of milk fat (% of total fatty acids) for cows fed the dietary treatments.

	Diet				
Fatty Acids	Control	Untreated	Formaldehyde treated	Heat treated	SEM
$C_{4:0}$	1.05	0.93	1.05	0.97	0.05
$C_{6:0}$	2.19	2.11	2.12	2.00	0.07
$C_{8:0}$	1.54^{ab}	1.60^{ab}	1.64^{a}	1.53^{b}	0.03
$C_{10:0}$	3.86	3.85	3.98	3.73	0.10
$C_{11:0}$	0.12	0.13	0.13	0.12	0.01
$C_{12:0}$	4.39	4.28	4.37	4.17	0.13
$C_{12:1}$	0.13^{a}	0.12^{ab}	0.10^{c}	0.11^{bc}	0.01
$C_{13:0}$	0.18	0.18	0.17	0.16	0.01
$C_{14:0}$	12.40	12.50	11.80	11.88	0.24
$C_{14:1}$	1.10^{a}	1.10^{a}	0.93^{b}	1.03^{ab}	0.05
anti-iso $C_{15:0}$	0.44^{ab}	0.47^{a}	0.43^{ab}	0.43^{b}	0.01
$C_{15:0}$	1.31	1.29	1.17	1.16	0.06
Total $C_{15:0}$	1.75	1.75	1.61	1.59	0.07

[a, b, c] Means in the same row with different superscripts differ ($P<0.05$)

Table 4.3. Least square means for carbon 16 and 17 fatty acids of milk fat (% of total fatty acids) for cows fed the dietary treatments.

	Diet				
Fatty Acids	Control	Untreated	Formaldehyde treated	Heat treated	SEM
iso $C_{16:0}$	0.27	0.28	0.27	0.25	0.01
$C_{16:0}$	32.67^{a}	26.98^{c}	26.79^{c}	29.35^{b}	0.79
Total $C_{16:0}$	32.94^{a}	27.26^{c}	27.06^{c}	29.60^{b}	0.79
$C_{16:1}$	1.60^{ab}	1.28^{bc}	1.21^{c}	1.62^{a}	0.10
$C_{17:0}$	0.91^{a}	0.79^{b}	0.77^{b}	0.83^{ab}	0.03
$C_{17:1}$	0.29^{a}	0.21^{b}	0.22^{b}	0.23^{b}	0.01

[a, b, c] Means in the same row with different superscripts differ ($P<0.05$)

the total $C_{18:1}$ was determined by totalling the *cis* and *trans* isomers. There was no significant increase in $C_{18:1}$ *trans* in milk fat for cows fed the canola seed treatments compared to the control. Milk from cows fed the untreated canola seed diet had numerically higher $C_{18:1}$ *trans* fatty acid, heat treated canola seed was intermediate and formaldehyde treated canola seed had the lowest percentage for the canola seed treatments but all were less than 1.75% of the fatty acids. Cows fed the canola seed treatments had a similar and increased concentration of $C_{18:1\omega 9}$ *cis* in milk when compared to the control. Cows fed the formaldehyde treated canola seed had the highest percentage of $C_{18:1\omega 7}$ *cis* in milk, whereas, similar percentages were noted for the other treatments. The overall total of $C_{18:1}$ in milk for the canola seed treatments was significantly different than in milk from the control.

Table 4.4. Least square means for carbon 18 fatty acids of milk fat (% of total fatty acids) for cows fed the dietary treatments.

	Diet				
Fatty Acid	Control	Untreated	Formaldehyde treated	Heat treated	SEM
$C_{18:0}$	9.92^{c}	13.14^{a}	11.10^{b}	11.73^{b}	0.27
$C_{18:1trans}$	1.24	1.77	1.44	1.65	0.09
$C_{18:1\omega 9\ cis}$	17.82^{b}	20.14^{a}	21.69^{a}	20.45^{a}	0.67
$C_{18:1\omega 7\ cis}$	0.71^{b}	0.66^{b}	0.85^{a}	0.61^{b}	0.03
Total $C_{18:1}$	19.77^{b}	22.57^{a}	23.98^{a}	22.70^{a}	0.68
$C_{18:2}$	1.96^{b}	1.98^{b}	3.45^{a}	2.05^{b}	0.06
$C_{18:3}$	0.51^{b}	0.57^{b}	1.14^{a}	0.57^{b}	0.03

$^{a, b, c}$ Means in the same row with different superscripts differ ($P<0.05$)

Linoleic acid ($C_{18:2}$) in milk (Table 4.4) was not affected by the inclusion of untreated or heat treated canola seed in the diet when compared to the control. Linoleic acid in milk from cows fed the formaldehyde treated canola seed diet was significantly greater than in milk from cows fed the other canola seed treatments and the control. The same trend for $C_{18:3}$ (Table 4.4), linolenic acid, was found as for $C_{18:2}$. Linolenic acid in milk from cows fed the control, untreated and heat treated canola seed were not significantly different from each other. Linolenic acid in milk from cows fed the formaldehyde treated canola seed diet was

significantly higher than in milk from cows fed the other canola seed treatments and the control.

Individual fatty acids of carbon chain length 20 or greater (Table 4.5) comprise less than 1% of the total fatty acids in milk. Significant differences were noted for $C_{20:1}$ and $C_{22:2}$. Peaks found in the chromatographs which could not be identified as a particular fatty acid were recorded as being unknown and significant differences were noted. Fourteen peaks were identified as being unknown fatty acids in milk. Elution times (min) for the unknown fatty acids are indicated in Table 4.5a. The retention times for these unknown fatty acids may help in determining their identification in milk fat. Untreated canola seed fed cows had the highest percentage of unknown fatty acids in milk with the formaldehyde treated canola seed fed cows having the lowest concentration.

The fatty acid composition of milk was also examined in terms of the percentage of monounsaturated, polyunsaturated and saturated fatty acids in milk (Table 4.6). The addition of untreated canola seed did not result in an increase in the percentage of monounsaturated fatty acids in milk. The formaldehyde treated and heat treated canola seed fed cows had increased concentrations of monounsaturated fatty acids when compared to cows fed the control diet but were similar to cows fed the untreated canola seed treatment. The polyunsaturated fatty acid levels in milk were similar for the control, heat treated and untreated canola seed fed cows but were lower than cows fed the formaldehyde treated canola seed diet. The concentration of saturated fatty acids in milk decreased with the inclusion of the canola seed treatments compared to the control. The percentage of saturated fatty acids in milk was not significantly different for cows fed untreated and heat treated canola seed diets. Cows fed formaldehyde treated canola seed had the lowest percentage ($P<0.05$) of saturated fats in milk.

The percentage of $C_{12:0} + C_{14:0} + C_{16:0}$ in milk declined with the inclusion of canola seed (Table 4.6). The formaldehyde treated canola seed treatment resulted in the lowest level of $C_{12:0} + C_{14:0} + C_{16:0}$ fatty acids in milk but was similar to cows fed the untreated canola seed diet. The ratio of $(C_{18:0} + C_{18:1})/C_{16:0}$ in milk increased with the inclusion of canola seed when compared to the control. Cows fed the untreated canola seed diet had the highest percentage

of $(C_{18:0} + C_{18:1})/C_{16:0}$ in milk but was similar to cows fed formaldehyde treated canola seed. Milk from cows fed heat treated canola seed had the lowest percentage of $(C_{18:0} + C_{18:1})/C_{16:0}$ of the three canola seed treatments.

No significant differences were observed for short-chain fatty acids in milk when the

Table 4.5. Least square means for carbon 20 and 22 fatty acids of milk fat (% of total fatty acids) for cows fed the dietary treatments.

	Diet				
Fatty Acids	Control	Untreated	Formaldehyde treated	Heat treated	SEM
$C_{20:1}$	0.18^{ab}	0.21^{a}	0.17^{b}	0.20^{a}	0.01
$C_{20:2}$	0.01	0.01	0.04	0.01	0.01
$C_{20:3}$	0.06	0.04	0.05	0.06	0.01
$C_{20:4}$	0.12	0.11	0.11	0.12	0.01
$C_{22:0}$	0.06	0.08	0.05	0.05	0.01
$C_{20:5}$	0.07	0.05	0.07	0.05	0.01
$C_{22:2}$	0.21^{a}	0.18^{ab}	0.21^{a}	0.13^{b}	0.02
$C_{22:5}$	0.09	0.09	0.09	0.09	0.01
$C_{22:6}$	0.05	0.03	0.04	0.03	0.01
Unknown	2.28^{bc}	2.63^{a}	2.12^{c}	2.50^{ab}	0.08

[a, b,c] Means in the same row with different superscripts differ ($P<0.05$)

Table 4.5a. Retention times for the unknown fatty acids in milk fat.

Fatty Acid	Retention time (min)	Fatty Acid	Retention time (min)
x1	4.84	x8	8.94
x2	4.90	x9	9.04
x3	5.35	x10	9.15
x4	7.33	x11	10.09
x5	7.47	x12	10.65
x6	8.04	x13	11.36
x7	8.81	x14	14.26

Table 4.6. Least square means for the fatty acid composition of milk fat (%) for cows fed the dietary treatments.

	Diet				
Fatty Acids	Control	Untreated	Formaldehyde treated	Heat treated	SEM
Monounsaturated[1]	23.07^{b}	25.49^{ab}	26.61^{a}	25.89^{a}	0.73
Polyunsaturated[2]	3.08^{b}	3.04^{b}	5.19^{a}	3.11^{b}	0.10
Saturated[3]	71.32^{a}	68.61^{b}	65.84^{c}	68.37^{b}	0.77
$C_{12:0}+C_{14:0}+C_{16:0}$	49.46^{a}	43.76^{bc}	42.96^{c}	45.40^{b}	0.79
$(C_{18:0}+C_{18:1})/(C_{16:0})$	0.93^{c}	1.34^{a}	1.32^{a}	1.16^{b}	0.05

$^{a, b,c}$ Means in the same row with different superscripts differ ($P<0.05$),[1] $C_{12:1}$, $C_{14:1}$, $C_{16:1}$, $C_{17:1}$, $C_{18:1t9trans}$, $C_{18:1t12}$, $C_{18:1t9cis}$, $C_{18:1t7cis}$, $C_{20:1}$,[2] $C_{18:2}$, $C_{18:3}$, $C_{20:2}$, $C_{20:3}$, $C_{20:4}$, $C_{20:5}$, $C_{22:2}$, $C_{22:5}$, $C_{22:6}$, [3] $C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{11:0}$, $C_{12:0}$, $C_{13:0}$, $C_{14:0}$, anti-iso $C_{15:0}$, $C_{15:0}$, iso $C_{16:0}$, $C_{16:0}$, $C_{16:1}$, iso $C_{17:0}$, $C_{17:0}$, $C_{18:0}$, $C_{22:0}$

cows were fed the dietary treatments (Table 4.7). Cows fed the formaldehyde treated canola seed had the lowest level of medium-chain fatty acids in milk when compared to the other treatments, however, the percentage was similar to cows fed the untreated canola seed diet. The percentage of long-chain fatty acids in milk increased with the inclusion of canola seed. Cows fed the formaldehyde treated canola seed had the highest percentage of long-chain fatty acids in milk but was not significantly different from cows fed the untreated canola seed diet.

The composition of FFA in milk was also analyzed (Table 4.8). No significant differences in FFA in milk were found for $C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:1}$ and $C_{18:2}$, however, $C_{18:0}$ was highest in milk from cows fed the heat treated canola seed diet compared to milk from cows fed the formaldehyde treatment.

4.3.3. Milk Protein Fractional Composition

No significant differences were found for CP, NPN or whey protein when these values

Table 4.7. Least square means for the fatty acid composition of milk fat (%) for cows fed the dietary treatments.

	Diet				
Fatty Acids	Control	Untreated	Formaldehyde treated	Heat treated	SEM
Short-chain[1]	13.28	13.03	13.39	12.63	0.29
Medium-chain[2]	50.99[a]	44.89[bc]	43.59[c]	46.78[b]	0.80
Long-chain[3]	33.01[c]	39.03[ab]	40.49[a]	37.80[b]	0.80

[a, b, c] Means in the same row with different superscripts differ (P<0.05), [1] $C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{11:0}$, $C_{12:0}$, $C_{12:1}$, [2] $C_{14:0}$, $C_{14:1}$, anti-iso $C_{15:0}$, $C_{15:0}$, iso $C_{16:0}$, $C_{16:0}$, $C_{16:1}$, iso $C_{17:0}$, $C_{17:0}$, $C_{17:1}$, [3] $C_{18:0}$, $C_{18:1t9trans}$, $C_{18:1t12}$, $C_{18:1t9cis}$, $C_{18:1t7}$, $C_{18:2}$, $C_{18:3}$, $C_{20:1}$, $C_{20:2}$, $C_{20:3}$, $C_{20:4}$, $C_{22:0}$, $C_{20:5}$, $C_{22:2}$, $C_{22:5}$, $C_{22:6}$

Table 4.8. Least square means for free fatty acids (FFA) of milk for cows fed the dietary treatments.

	Diet				
FFA, mg/ppm	Control	Untreated	Formaldehyde treated	Heat treated	SEM
$C_{4:0}$	4.388	4.733	1.178	8.550	2.61
$C_{6:0}$	3.002	3.204	1.035	5.813	1.70
$C_{8:0}$	2.625	3.149	1.232	4.336	1.05
$C_{10:0}$	4.421	5.388	2.132	7.144	1.70
$C_{12:0}$	5.118	5.784	3.101	7.552	1.53
$C_{14:0}$	9.506	10.468	4.958	14.213	2.92
$C_{16:0}$	24.878	25.475	16.418	31.384	5.04
$C_{16:1}$	1.858	1.753	1.135	2.323	0.46
$C_{18:0}$	10.482[ab]	13.579[ab]	8.435[b]	16.156[a]	2.19
$C_{18:1}$	23.573	29.724	17.668	38.811	6.41
$C_{18:2}$	1.559	2.330	1.625	3.417	0.62

[a, b] Means in the same row with different superscripts differ (P<0.05)

were expressed as a percentage of milk (Table 4.9). True protein and casein protein fractions expressed as a percentage of milk were altered by the dietary treatments. Differences were observed for the true protein and casein protein fractions between cows fed the untreated and formaldehyde treated canola seed but not for cows fed the other dietary treatments. True protein, casein protein, NPN and whey protein fractions expressed as a percentage of CP were not affected by diet. Milk true protein is composed of two fractions, the casein proteins (alpha, kappa and beta) and the whey proteins (lactoglobulin and lactalbumin). Table 4.9 illustrates the percentage of casein and whey proteins which were found in milk protein. Data from this milk analysis illustrated that the kappa and alpha$_{s1}$ casein proteins in milk were not affected by dietary treatment, however, the dietary treatments significantly affected the beta casein fraction of milk protein. Lactalbumin in milk from cows fed the formaldehyde treated canola seed diet was significantly lower from the cows fed the other treatments. No significant differences were found in the percentage of lactoglobulin in milk. A portion of the whey proteins which could not be identified were labelled unknown. Cows fed the formaldehyde treated and heat treated canola seed diets exhibited differences for unknown whey proteins in milk, however, no other treatments differences were noted.

4.3.4. Mineral Composition of Milk

The mineral analysis of milk is shown in Table 4.10. Mineral results are expressed in terms of whole fluid milk as either mg/100 g or μg/100 g for macro-minerals or micro-minerals, respectively. There were no significant differences found in the macro-minerals, which include Ca, Mg, S, K, P and Na. No statistical differences were found for the following micro-minerals - Al, Cu, Mn or Zn. Iron and B were, however, significantly different as a result of the dietary treatments. The concentration of Fe in milk was similar for cows fed the control, untreated and formaldehyde treated diets and cows fed the untreated canola seed was similar to cows fed the heat treated canola seed diet. Cows fed the untreated and heat treated canola seed diets had significantly higher concentrations of B in milk compared to cows fed the control and formaldehyde treated canola seed diets.

Table 4.9. Least square means for fractional composition of milk protein for cows fed the dietary treatments.

Item	Diet				SEM
	Control	Untreated	Formaldehyde treated	Heat treated	
	% of milk				
Crude Protein	3.13	3.15	3.05	3.11	0.07
True Protein	2.86^{ab}	2.93^{a}	2.80^{b}	2.88^{ab}	0.04
Casein Protein	2.39^{ab}	2.45^{a}	2.29^{b}	2.36^{ab}	0.03
NPN	0.27	0.22	0.25	0.23	0.05
Whey Protein	0.47	0.48	0.50	0.52	0.03
	% of CP				
True Protein	91.43	92.81	91.83	92.54	1.49
Casein Protein	76.38	77.67	75.29	75.67	1.11
NPN	8.58	7.13	8.16	7.46	1.51
Whey Protein	15.04	15.18	16.58	16.96	0.99
	mg/ml of milk				
Casein Proteins					
Kappa	2.80	2.83	2.72	2.81	0.10
Alpha$_{s1}$	9.58	9.59	9.19	9.40	0.13
Beta	9.92^{ab}	10.32^{a}	9.37^{b}	9.68^{b}	0.17
Whey Proteins					
Lactalbumin	0.87^{a}	0.95^{a}	0.67^{b}	0.99^{a}	0.04
Lactoglobulin	3.33	3.40	3.18	3.37	0.10
Unknown	0.17^{ab}	0.18^{ab}	0.13^{b}	0.19^{a}	0.02

$^{a, b}$ Means in the same row with different superscripts differ ($P<0.05$)

Table 4.10. Least square means for the mineral composition of whole fluid milk for cows fed the dietary treatments.

Mineral	Diet				SEM
	Control	Untreated	Formaldehyde treated	Heat treated	
Macro-minerals, mg/100g					
Calcium	127.65	124.67	120.50	104.45	10.50
Magnesium	10.31	10.77	10.36	10.08	0.20
Sulphur	31.97	31.70	30.12	27.86	1.43
Potassium	125.10	139.05	117.95	135.29	6.18
Phosphorous	99.11	100.56	92.69	87.05	5.57
Sodium	30.99	34.08	34.62	36.06	1.41
Trace minerals, μg/100g					
Iron	29.25^{ab}	18.26^{bc}	36.67^{a}	15.31^{c}	3.75
Aluminum	35.99	38.09	30.60	34.78	4.48
Copper	7.96	6.94	8.44	6.73	0.76
Manganese	1.77	1.66	1.51	1.66	0.15
Boron	14.91^{b}	19.19^{a}	13.30^{b}	20.48^{a}	1.13
Zinc	423.09	414.14	354.33	353.60	47.81

[a, b, c] Means in the same row with different superscripts differ ($P<0.05$)

4.5. Discussion

4.5.1. Cow Performance

Body weight and BCS were not influenced by dietary treatment suggesting that the diets were readily consumed by the dairy cows. Research has both shown increases, decreases or no body weight changes when dietary fat was added to the diet. Markus et al. (1996), Salfer et al. (1995) and Wu et al. (1994) all found that body weight was unaffected with the addition of dietary fat in the diet. Dry matter (DM) intake was not affected by the inclusion of canola seed in the diet.

Daily milk yield was similar for the dietary treatments. Addition of canola seed, either unprotected or protected with heat or formaldehyde, in other studies resulted in milk yields

being similar among dietary treatments (Khorasani and Kennelly, 1996, Ashes et al., 1992, Handy and Kennelly, 1983). Others have found that the addition of various dietary fat sources has caused significant increases in milk yield as much as 10% or greater (Kim et al., 1993, Murphy and Connolly, 1991, Schingoethe and Casper, 1991). Khorasani et al. (1991), Kennelly et al. (1987) and Kennelly (1983) reported that increasing the level of canola seed up to 4.5% (of dietary DM) in the diet increased milk yield. Schauff and Clark (1992) suggested supplemental fat is of greatest benefit when cows yield greater than 35 kg of milk per day. It is possible that the similarity in milk production in this study is due to the fact that the average milk production was less than 35 kg/day (33.3 ± 1.57kg/day). Khorasani et al. (1991) reports the stage of lactation and the level of milk production are important factors moderating responses to dietary fat. When animals are in neutral or positive energy balance a positive response in milk yield is less likely to occur with the inclusion of fat.

No significant differences were found for daily milk fat yield which is in agreement with other studies utilizing canola seed as a dietary fat source (Khorasani and Kennelly, 1996, Murphy et al., 1990, Handy and Kennelly, 1983). Ashes et al. (1992) used formaldehyde protected canola seed and observed significant increases in milk fat yield at an inclusion level of 6.5% of the total ration offered (520 g/day). Ashes et al. (1992) states feeding oils encapsulated in an aldehyde-treated protein matrix results in an increase in milk fat yield which is indicative of a supplement reasonably well protected from rumen degradation. The process utilized in the 1992 study was used to create the formaldehyde treated canola seed for the current study, however, no significant increases in milk fat yield were observed. It is possible that not all formaldehyde treated supplements produce the same result or that the inclusion level of fat (approximately 2.1% added fat) was not large enough to affect milk fat in this study. Khorasani et al. (1991) found with increasing increments of heat treated canola seed, milk fat yield increased up to 4.5% canola seed in the diet and declined with further increases of canola seed. Jenkins and Jenny (1992) and Kennelly et al. (1987) indicated addition of dietary fat caused a decline in milk fat yield. Several studies found that the inclusion of canola seed had no effect on milk fat percentage (Khorasani and Kennelly, 1996, 1995, Khorasani et al., 1991). Studies have found that milk fat percentage declined with the

inclusion of supplemental dietary fat (Atwal et al., 1991, Schingoethe and Casper, 1991, Astrup et al., 1976). Others indicate that adding various dietary fat sources to the diet causes an increase in milk fat percentage which is in contrast with the present study (Ashes et al., 1992, Wrenn et al., 1977, Bitman et al., 1975). No differences were found in DM intake, fermentation characteristics or acetate:propionate ratios for the dietary treatments, suggesting that the lower milk fat content of cows fed the untreated canola seed diet may not be related to fermentation characteristics. A decline in milk fat has been attributed to fat supplementation interfering with rumen fermentation, fibre digestibility, lower acetate:propionate ratios or the degree of saturation. Khorasani et al. (1991) states the effect of supplemental fat on milk fat percentage depends upon the balance between decreased intramammary synthesis of short- and medium-chain fatty acids and on the extent of incorporation of additional dietary long-chain fatty acids into milk fat.

A decrease in milk protein is a concern since it is important to the nutritive value of most dairy products (ie: cheese making) (Dunkley et al., 1977). Milk protein yield and percentage were similar for each of the dietary treatments illustrating the addition of canola seed did not depress protein yield or percentage. This contradicts the general observation that supplemental fat decreases milk protein concentration (Grummer et al., 1994, Khorasani and Kennelly, 1994, Wu et al., 1994). Added dietary fat can result in a decrease in milk protein concentration due to a restriction in amino acid availability for milk protein synthesis in the mammary gland (Wu et al., 1994), reduced availability of glucose (Smith et al., 1978) or lowered plasma insulin (Johnson et al., 1988). Other studies have found that dietary fat sources, either unprotected or protected, did not cause any differences in protein yield (Khorasani and Kennelly, 1996, Salfer et al., 1995, Ashes et al., 1992). Ashes et al. (1992) found that formaldehyde treated canola seed had a similar protein yield as the control which was also observed in the current study. Khorasani and Kennelly (1996) and Khorasani et al. (1991) found that increasing the level of heat treated canola seed did not influence protein yield. Supplemental fat, however, can depress protein yield as was illustrated by Elliott et al. (1993), Jenkins and Jenny (1992) and Mielke and Schingoethe (1981). No differences in milk protein percentage were observed when formaldehyde and heat treated canola seed sources

were utilized (Khorasani and Kennelly, 1996, Ashes et al., 1992, Emanuelson et al.,1991).

Lactose yield and percentage in the present study did not show any significant differences among treatments, however, lactose percentages were lower than the normal percentage of 4.80-5.0% suggested by Kennelly (1996a, 1995) and Brink and Lofgren (1982). No significant differences were noted for feed intake, propionic acid production, milk protein content or bacterial yield and composition which can influence lactose production. Smith et al. (1978) suggests a decline in milk lactose content is due to altered glucose metabolism from added dietary fat, however, glucose metabolism was not studied and can not be confirmed that glucose metabolism was altered by the inclusion of dietary fat. Lactose concentrations are relatively constant (Kennelly, 1996a, Sutton, 1989) and data for the current study illustrates similar concentrations and yields for lactose for the dietary treatments. Addition of canola seed, either unprotected or protected, did not result in any differences in lactose yield or percentage (Khorasani and Kennelly, 1996, Kennelly, 1983, Handy and Kennelly, 1983). Addition of dietary fat has caused lactose yields to decline (Kennelly, 1983, MacLeod et al., 1977, Pan et al., 1972). Khorasani et al. (1991) observed that heat treated canola seed caused lactose yield to increase quadratically up to 4.5% canola seed (dietary DM) where it reached a maximum and declined with increasing levels canola seed.

4.5.2. Milk Fatty Acid Composition

4.5.2.1. Short-Chain Fatty Acids in Milk Fat

A variety of dietary fat sources have been utilized by researchers to manipulate the fatty acid composition in milk. The literature review described the biosynthesis and factors affecting milk fatty acid composition and there are various excellent reviews which discuss these subjects (Kennelly, 1996a, Palmquist et al., 1993, Grummer, 1991, Moore and Christie,1981).

Concentrations of butyric acid ($C_{4:0}$), caproic acid ($C_{6:0}$), capric acid ($C_{10:0}$) and lauric acid ($C_{12:0}$) in milk fat did not show any significant differences when canola seed was added to the diet. This is in agreement with other research work (Khorasani and Kennelly, 1996, Ashes et al., 1992, Emanuelson et al., 1991). Some studies have found significant differences

in the concentration of these fatty acids in milk fat with the addition of dietary fat (Kim et al., 1993, Khorasani et al., 1991, DePeters et al., 1989). Palmquist and Jenkins (1980) suggest the concentration of $C_{4:0}$ may not differ among treatments as $C_{4:0}$ can probably be synthesized by routes other than the malonyl CoA pathway. Jensen et al. (1991) states that the analysis of bovine milk is difficult due to problems concerning loss or separation of short-chain fatty acids. The concentration of caprylic acid ($C_{8:0}$) in milk fat was significantly altered with the addition of canola seed which is in agreement with Murphy et al. (1990) and Kennelly et al. (1987). Khorasani and Kennelly (1996) and Ashes et al. (1992) found no significant differences for $C_{8:0}$ concentrations. Atwal et al. (1991), however, found that feeding formaldehyde protected canola seed significantly decreased $C_{8:0}$, however, formaldehyde treatment did not influence $C_{8:0}$ for the current study.

When $C_{4:0}$ to $C_{12:0}$ fatty acids in milk are classified as the short-chain fatty acids, no significant differences were observed with the addition of approximately 2.1% fat to the diet. Addition of dietary fat in other studies has demonstrated that short-chain fatty acids in milk decline. Jenkins and Jenny (1992) and Grummer (1991) indicate that de novo synthesis of $C_{6:0}$ to C_{16} fatty acids in the mammary gland decreases when supplemental fats are fed to dairy cows. Palmquist and Jenkins (1980) state that de novo synthesis is apparently inhibited by reduced activity of acetyl CoA carboxylase (ACC) from the increased supply of preformed fatty acids. Using canola seed as a supplemental fat source has resulted in a significant decline in the content of short-chain fatty acids in milk fat (Khorasani and Kennelly, 1996, Atwal et al., 1991, Murphy et al., 1990). A number of studies conducted at the University of Alberta to evaluate the effectiveness of whole canola seed as both a fat and protein source for lactating dairy cows have found that milk from cows fed ground canola seed or canola oil resulted in significantly lower concentrations of short-chain fatty acids (Kennelly, 1983, Kennelly and Fenton, 1982).

Grummer (1991) indicates a reduction in short-chain fatty acids and medium-chain fatty acids has been attributed to (1) a reduction in the ruminal production of acetate and butyrate, which are two precursors for de novo synthesis of these fatty acids in the mammary gland and (2) a dilution or direct inhibitory effect of dietary long-chain fatty acids. No

significant differences, however, were observed for the concentrations of acetate and butyrate in rumen fluid for any of the dietary treatments. No differences in volatile fatty acid (VFA) concentrations were observed for this study, therefore, based upon Grummer's (1991) statement no affect on the concentration of short-chain fatty acids should be observed. This was found to be true for the current study.

4.5.2.2. Medium-Chain Fatty Acids in Milk Fat

Myristic acid ($C_{14:0}$) was not influenced by inclusion of canola seed in the diet. Cows fed both the formaldehyde and heat treated canola seed, however, did have numerically lower $C_{14:0}$ in milk fat. Addition of canola seed in other studies has indicated that canola seed did not influence the concentration of $C_{14:0}$ in milk when inclusion levels did not exceed 5% of dietary DM (Khorasani and Kennelly, 1996, Emanuelson et al., 1991). Khorasani and Kennelly (1996) found increasing the level of heat treated canola seed in the diet greater than 3.75% (dietary DM) resulted in a linear decline in $C_{14:0}$. Ashes et al. (1995) and Atwal et al. (1991) both found that formaldehyde protected canola seed caused a significant decline in $C_{14:0}$.

The concentration of palmitic acid will be discussed as the total of iso $C_{16:0}$ and $C_{16:0}$ and will be either stated as total $C_{16:0}$ or $C_{16:0}$. A significant depression occurred in the content of $C_{16:0}$ due to the inclusion of canola seed in the diet. Inclusion of the canola seed resulted in a 17.9, 17.2 and 10.2% decline for cows fed the formaldehyde treated, untreated and heat treated canola seed diets, respectively. Palmitic acid is derived from dietary fat sources, adipose tissue and the diet via the fermentation of carbohydrates to produce the precursors of acetate and β-hydroxybutyrate which are derived from acetic and butyric acids. The concentrations of both acetate and butyrate in rumen fluid were not affected by the inclusion of canola seed. The fatty acid composition of the heat treated canola seed diet had similar values as the untreated canola seed diet (Table 2.1). Long-chain fatty acids from dietary fat sources and body stores of fat can also contribute to the concentration of $C_{16:0}$ and could have influenced its concentration in milk fat. The heat treated canola seed diet resulted in decreasing the content of $C_{16:0}$ when compared to the control but was not as effective as the

untreated or formaldehyde treated canola seed diets.

A high concentration of $C_{16:0}$ and $C_{14:0}$ in milk continues to be a concern to the medical field and the medical community (Kennelly, 1996b). Kennelly (1996b) reports that dietary manipulation which results in a decline in $C_{16:0}$ and $C_{14:0}$ in milk fat and results in an increase in $C_{18:0}$ and $C_{18:1}$ fatty acids in milk is considered desirable from a human health perspective. An increase in $C_{18:0}$ and $C_{18:1}$ fatty acids was observed in this study with a decline in the levels of $C_{16:0}$ and $C_{14:0}$. Other studies which have incorporated canola seed into the diet have also observed significant declines in $C_{16:0}$. Khorasani and Kennelly (1996) and Khorasani et al. (1991) found both linear and quadratic declines, respectively, in the concentration of $C_{16:0}$. Several research studies utilizing formaldehyde treated canola seed also observed a significant decline in $C_{16:0}$ (Ashes et al., 1995, 1992, Atwal et al., 1991).

The $C_{14:0}$ to $C_{17:1}$ fatty acids make up the medium-chain fatty acids. In comparison to the control diet, each of the canola seed treatments caused significant declines in medium-chain fatty acids in milk fat. When the two protective methods used to prevent biohydrogenation in the rumen are compared, milk from the formaldehyde treated canola seed fed cows caused the greatest depression in medium-chain fatty acids compared to heat treated canola seed fed cows. Inclusion of canola seed in the diet of the lactating dairy cow resulted in medium-chain fatty acids in milk fat declining 14.5, 12 and 8.3% for cows fed formaldehyde treated, untreated and heat treated canola seed, respectively, when compared to control cows. A decrease in medium-chain fatty acids in milk fat with the inclusion of heat or formaldehyde treated canola seed is in agreement with others (Khorasani and Kennelly, 1996, Atwal et al., 1991). Other studies have reported a decline in medium-chain fatty acids in milk without using protected canola seed but rather using increasing levels of whole canola seed or ground canola seed (Murphy et al., 1990, Kennelly et al., 1987, Kennelly and Fenton, 1982). Palmquist and Jenkins (1980) reported that reductions in short- and medium-chain fatty acids in milk fat may have resulted from lower production of acetate and β-hydroxybutyrate in the rumen or may have resulted from increased uptake of dietary fatty acids by the mammary gland which probably inhibited de novo synthesis of short-chain fatty acids. As previously stated, acetate and butyrate concentrations in rumen fluid were not

significantly different among the dietary treatments. It has also been stated that de novo synthesis of short- and medium-chain fatty acids is decreased via decreased activity of ACC resulting from an increased supply of preformed fatty acids. In summary, this study demonstrated that manipulating the diet of the dairy cow with the addition of canola seed can alter the balance of de novo synthesis of short- and medium-chain fatty acids found in milk fat.

4.5.2.3. Long-Chain Fatty Acids in Milk Fat

Appendix 4.5 illustrates transfer efficiencies of oleic, linoleic and linolenic acids in milk fat for cows fed the canola seed treatments. The content of stearic acid ($C_{18:0}$) in milk fat had significantly increased with the inclusion of canola seed when compared to the control. Milk fat from cows fed the untreated canola seed had the greatest increase in $C_{18:0}$ (32.5%) when compared to the control. This demonstrates that a larger proportion of unprotected canola seed did not escape rumen biohydrogenation resulting in a greater proportion of the dietary fatty acids being converted to $C_{18:0}$. Milk fat from cows fed formaldehyde treated and heat treated canola seed also resulted in increases in $C_{18:0}$ of 11.9 and 18.3%, respectively. The control demonstrated that diets containing no added fat produce lower levels of $C_{18:0}$ than those diets which contain protected or unprotected canola seed. Studies utilizing heat or formaldehyde treated canola seed in the diet of lactating dairy cows found an increase in $C_{18:0}$ in milk fat when compared to the control (Khorasani and Kennelly, 1996, Ashes et al., 1995, 1992). Research work not incorporating protected canola seed also reported increased proportions of $C_{18:0}$ in milk fat (Murphy and Connolly, 1991, Murphy et al., 1990, Handy and Kennelly, 1983). In heat treated canola seed studies by Khorasani and Kennelly (1996) and Khorasani et al. (1991) increases in $C_{18:0}$ at the lowest levels of canola seed inclusion (4.5 and 3.75%, respectively) saw increases of 40 and 45.2%, respectively. Ashes et al. (1992) reported an increase in $C_{18:0}$ and a decrease in $C_{16:1}$, which was also observed for the current study. These changes may have been due to inhibition of Δ9-desaturase enzyme that converts $C_{16:0}$ to $C_{16:1}$ and $C_{18:0}$ to $C_{18:1}$ in the mammary gland. Mahfouz et al. (1980) suggests that *trans* C_{18} fatty acids are potent inhibitors of the Δ9- microsomal desaturase enzyme systems

in the liver. Banks et al. (1990) suggests that the amount of *trans* $C_{18:1}$ fatty acid reaching the mammary gland significantly influences the amount and type of fatty acids synthesized in this tissue. The increases found in $C_{18:0}$ could have resulted from enzyme inhibition as suggested.

Oleic acid ($C_{18:1}$) was determined both as a total of the *cis* and *trans* isomers and in terms of the individual isomers. Cows fed the formaldehyde treated, heat treated and untreated canola seed saw increases in $C_{18:1}$ of 21.3, 14.8 and 14.2%, respectively. Studies incorporating canola seed into the diet of lactating dairy cows also demonstrated an increase in $C_{18:1}$ (Khorasani and Kennelly, 1996, Ashes et al., 1995, 1992). In agreement with the present trial, Emanuelson et al. (1991) found no significant differences in the content of $C_{18:1}$ between heat and unheated sources. Khorasani et al. (1991) reported that an increased concentration of $C_{18:1}$ supports the fact that canola seed escaped rumen hydrogenation. Grummer (1991) reports that increased $C_{18:1}$ in milk fat may have occurred because of greater desaturation of $C_{18:0}$ in the mammary gland to maintain proper fluidity of milk fat when short-chain fatty acids were decreased. This statement can apply to the current study since short-chain fatty acid composition was decreased and $C_{18:1}$ was increased.

The *cis* and *trans* isomers of $C_{18:1}$ have been identified in some studies that have incorporated canola seed in the lactating dairy cow diet. Grummer (1991) has stated that similar increases in the content of $C_{18:1}$ can be obtained with a wide variety of lipid supplements and the positional isomers of $C_{18:1}$ present may vary. The process of biohydrogenation of polyunsaturated fatty acids includes an intermediate step where *trans* fatty acids are produced (Jenkins, 1993, Scott and Ashes, 1993). Moore and Christie (1984) illustrate the biohydrogenation of linoleic and linolenic acids in Figure 4.1. For the *cis* isomers identified in the current study, all demonstrated significant differences among the dietary treatments, however, no significant differences were observed for the *trans* isomers identified. Increases in the concentration of isomers has been identified by authors which utilized protected canola seed (Ashes et al., 1995, Atwal et al., 1991, Handyand Kennelly, 1983) and unprotected canola seed (Kennelly et al., 1987, Handy and Kennelly, 1983,

Figure 4.1. Biohydrogenation of linoleic and linolenic acids.

Linoleic acid (C18:2)	Linolenic acid (C18:3)
cis 9, *cis* 12 18:2	*cis* 9, *cis* 12, *cis* 15 18:3
↓ isomerization	↓ isomerization
cis 9, *trans* 11 18:2	*cis* 9, *trans* 11, *cis* 15 18:3
↓ +2H	↓ +2H
trans 11 18:1	*trans* 11, *cis* 15 18:2
↓ +2H	↓ +2H
C18:0	*trans* 11 18:1
(stearic acid)	↓ +2H
	C18:0
	(stearic acid)

Kennelly and Fenton, 1982). Wu et al. (1991) reported increased production of *trans* fatty acids is due to the biohydrogenation of long-chain fatty acids in the rumen. Palmquist and Jenkins (1980) state *trans* $C_{18:1}$ isomers are normal intermediates in the biohydrogenation of $C_{18:2}$ and $C_{18:3}$ in the rumen. The formation of *trans* isomers in this study, therefore, demonstrates that some biohydrogenation did take place in the rumen. Grummer (1991) reports the ramifications of increasing *trans*-11 $C_{18:1}$ content on the nutritional value of milk is unknown, however, *trans* fatty acids are thought to behave like saturated fatty acids. Research, however, has focused on the major *trans* isomer, trans- 9 $C_{18:1}$.

Enig (1993) reports ruminant *trans* fatty acids are always accompanied by adequate levels of saturated fatty acids and they do not adversely affect the essential fatty acid status. This research review indicates intake of high levels of *trans* fatty acids can have adverse effects in both humans and animals (ie: atherosclerosis, cancer, diabetes, obesity) and cause undesirable effects on immune function, reproduction and lactation. The content of *trans* $C_{18:1}$ does not comprise a large percentage of the total $C_{18:1}$, less than 1.75%. Kennelly (1996b) indicates an advantage of feeding protected lipid supplements is that *trans* fatty acids would be avoided. The current study did not result in the *trans* fatty acids being avoided in milk fat as they were still detected. The advantage of using a protected lipid, however, was a reduction in the concentration of *trans* fatty acids present in milk fat when compared to milk fat from cows fed an unprotected lipid source.

In general, the addition of fat supplementation resulted in an increase in the *cis*

isomers of $C_{18:1}$ which make up the majority of the total $C_{18:1}$ fatty acids. This study demonstrated that dietary manipulation resulted in an increase in $C_{18:0}$ and $C_{18:1}$ fatty acids at the expense of $C_{16:0}$ and $C_{14:0}$ which is consistent with various other research studies. Dietary manipulation causing an increase in $C_{18:0}$ and $C_{18:1}$ fatty acids at the expense of $C_{16:0}$ and $C_{14:0}$ is considered to be desirable from a human health perspective (Kennelly 1996b). Also, an increase in $C_{18:0}$ and $C_{18:1}$ and a decline in $C_{14:0}$ and $C_{16:0}$ results in a softer butter (Kennelly, 1996b, Cadden et al., 1984). The fatty acids $C_{18:0}$ and $C_{18:1}$ were once targeted as being undesirable from a human health perspective but are now considered to have a neutral or positive effect on human health (Kennelly 1996b). Jensen et al. (1991) also reported that $C_{18:0}$ has been shown to be as effective as $C_{18:1}$ in reducing plasma cholesterol.

Linoleic acid ($C_{18:2}$) in milk originates almost entirely from the diet (Wrenn et al., 1976), however, body fat stores can be converted to produce this fatty acid. Addition of formaldehyde treated canola seed resulted in an increase in $C_{18:2}$ of 76%. The concentration of $C_{18:2}$ for cows fed heat treated canola seed was not significantly different from cows fed the control and untreated canola seed. Khorasani et al. (1991) reports an absence of a significant effect on $C_{18:2}$ suggests partial hydrogenation in the rumen. The formaldehyde treated canola seed proved to be more effectively protected from biohydrogenation in the rumen since a significant increase was observed for $C_{18:2}$. Emanuelson et al. (1991) and Khorasani et al. (1991) observed no significant effect on $C_{18:2}$ with the application of heat treatment. A recent study by Khorasani and Kennelly (1996) observed a linear increase in the concentration of $C_{18:2}$ with increasing levels of heat treated canola seed above 4.5% of dietary DM. Studies by Ashes et al. (1995, 1992) and Atwal et al. (1991) observed a significant increase in the content of $C_{18:2}$, whereas, the study by Murphy et al. (1990) reported a decline in $C_{18:2}$. These researchers stated that no increase in the $C_{18:2}$ content of milk was indicative of lipolysis and hydrogenation of the oil by rumen microbes.

The content of linolenic acid ($C_{18:3}$) was found to increase significantly with the inclusion of formaldehyde treated canola seed. The increase in $C_{18:3}$ was substantial (123.5%) for the formaldehyde treated canola seed diet in comparison to the heat treated canola seed diet (11.8%). Khorasani et al. (1991) and DePeters et al. (1985) indicated an absence of a

significant effect in the content of $C_{18:3}$, as well as $C_{18:2}$, suggesting partial hydrogenation of the oil by rumen microorganisms. The current study demonstrates that the heat treated canola seed was not as well protected from rumen hydrogenation as the formaldehyde treated canola seed. Studies incorporating formaldehyde treated canola seed observed a significant increase in $C_{18:3}$ in milk fat (Ashes et al., 1995, 1992, Atwal et al., 1991). These studies found $C_{18:3}$ in milk fat increased approximately 62 to over 100% when compared to the control. Khorasani and Kennelly (1996), Khorasani et al. (1991) and Emanuelson et al. (1991) observed no significant effects for the content of $C_{18:3}$ with increasing levels of heat treated canola seed. This is consistent with the results obtained for cows fed the heat treated canola seed diet in this experiment.

The fatty acids of carbon chain length 18 and greater are termed long-chain fatty acids. The ability of the mammary gland to incorporate dietary long-chain fatty acids into milk fat provides the opportunity to alter the content and type of fat present in milk fat (Kennelly, 1996a). As previously stated by Kennelly and Khorasani (1992), de novo synthesis in the mammary gland of short- and medium-chain fatty acids and dietary and microbial long-chain fatty acids presented to the mammary gland can be altered by dietary manipulation. Kennelly (1996a) states changes in long-chain fatty acids are probably a result of both a reduction in de novo synthesis of short- and medium-chain fatty acids and an increased uptake of dietary long-chain fatty acids. Supplementing the diet with canola seed resulted in a decline in both short- and medium-chain fatty acids with a subsequent increase in the long-chain fatty acids. The increase in long-chain fatty acids with the addition of canola seed in the diet is consistent with research of Khorasani and Kennelly (1996), Ashes et al. (1992) and Khorasani et al. (1991). The current study illustrated that cows fed formaldehyde treated canola seed incorporated a greater concentration of long-chain fatty acids into milk fat than heat treated or untreated canola seed fed animals. Increases in long-chain fatty acids were also observed in other studies which incorporated dietary fat sources in the lactating dairy cow diet (Markus et al., 1996, Kim et al., 1993, Middaugh et al., 1988). For the current study, long-chain fatty acids in milk fat increased by 14.5, 18.2 and 22.7% for cows fed untreated, heat treated and formaldehyde treated canola seed, respectively.

4.5.2.4. Monounsaturated, Polyunsaturated and Saturated Fatty Acids

From the results obtained for the concentration of monounsaturated, polyunsaturated and saturated fatty acids in milk, the formaldehyde treated canola seed treatment had the greatest impact, the heat treated canola seed treatment was intermediate and the untreated canola seed treatment had the lowest impact. The current study is in agreement with Khorasani and Kennelly (1996), Ashes et al. (1992) and Emanuelson et al., (1991).

In the rumen, biohydrogenation (reduction of double bonds) affects the fatty acids reaching the mammary gland. Long-chain fatty acids of dietary origin are hydrogenated to both saturated and monounsaturated fatty acids resulting in the polyunsaturated fatty acids (ie: $C_{18:2}$, $C_{18:3}$) being absent since they are hydrogenated to $C_{18:0}$ or a number of geometrical (*trans*) or positional isomers of the monounsaturated fatty acid $C_{18:1}$. This results in milk being relatively saturated compared to the diet of the dairy cow. The diets utilized clearly demonstrated that by not effectively protecting the dietary fat source from rumen biohydrogenation the polyunsaturated fatty acids remain relatively low and that monounsaturated and saturated fatty acids remain relatively high in milk. The heat treated and untreated canola seed fed cows generally produced similar results suggesting that the heat treated canola seed may not have been as well protected from rumen biohydrogenation as expected. The control which did not have an added fat source demonstrated that without supplying additional fat to the diet, that dietary components contribute to the fatty acid composition of milk fat. Also, body stores of fat contribute to the fatty acid composition of milk fat since adipose tissue can be utilized as a fat source.

Polyunsaturation of milk fat increased by 68.5% with the inclusion of formaldehyde treated canola seed. An increase in polyunsaturated fatty acids illustrates that the fat source was to some extent protected from rumen biohydrogenation. The increase in polyunsaturated fatty acids for cows fed the heat treated canola seed (1%) was very small or negligible suggesting that the fat source was not completely protected from rumen biohydrogenation. Increases in polyunsaturated fatty acids depends upon a number of factors such as the polyunsaturated fatty acid content of the fat source, level of inclusion, rumen biohydrogenation, protection level, diet or animal variation. Other research studies have

demonstrated that the content of polyunsaturated fatty acids in milk is in a range of approximately 2.5 to 10% depending on the experiment. An increase in polyunsaturated fatty acids is positive from a human health perspective since consumers desire products low in saturated fats.

Inclusion of formaldehyde treated and heat treated canola seed resulted in milk fat having a greater concentration of monounsaturated fatty acids compared to the control, however, concentrations were similar to the untreated canola seed treatment. The slight increases in the monounsaturated fatty acids, despite not being significant for the above treatments, resulted in decreasing the concentration of the saturated fatty acids in milk fat. An increase in the unsaturated fatty acids in milk fat resulted from the unsaturated nature of the fatty acids found in the canola seed (Khorasani et al., 1991) and the desaturase activity (conversion of $C_{18:0}$ to $C_{18:1}$) in the intestine and the mammary gland (Kennelly, 1996a). The addition of canola seed in the diet, therefore, results in improving the concentrations of monounsaturated and polyunsaturated fatty acids while reducing the level of saturated fatty acids.

4.5.2.5. $C_{12:0} + C_{14:0} + C_{16:0}$ and $C_{18:0} + C_{18:1}/C_{16:0}$ Fatty Acid Ratios

The addition of canola seed decreased the concentration of $C_{12:0} + C_{14:0} + C_{16:0}$ in milk fat in comparison to the control. Results from studies utilizing heat or formaldehyde treated canola seed were consistent with the decline found in the current experiment (Khorasani and Kennelly, 1996, Ashes et al., 1992, Atwal et al., 1991). Unprotected canola seed utilized by other researchers found a decline in the concentration of $C_{12:0} + C_{14:0} + C_{16:0}$ in milk fat (Khorasani and Kennelly, 1994, Murphy et al., 1990). The decline in these saturated fatty acids in milk fat illustrates that different fat sources and protection methods can reduce rumen biohydrogenation and influence the content of these fatty acids.

The ratio of $(C_{18:0} + C_{18:1})/C_{16:0}$ was observed to be low for the control diet, however, the addition of dietary fat in the form of canola seed resulted in significant increases in this ratio. Cows fed the untreated and formaldehyde treated canola seed had ratios which were not significantly different from each other. Cows fed the formaldehyde treated canola seed

did have a greater $(C_{18:0} + C_{18:1})/C_{16:0}$ ratio than the heat treated canola seed fed cows. The difference in the ratio for the heat treated canola seed diet could be related to the relatively high concentration of $C_{16:0}$ or that a greater portion of the lipid did not escape biohydrogenation. Studies which have utilized protected canola seed observed an increase in the ratio of $(C_{18:0} + C_{18:1})/C_{16:0}$ (Khorasani and Kennelly, 1996, Ashes et al., 1995, 1992). Feeding dairy cows unprotected canola seed also found an increase in this ratio in milk fat (Murphy et al., 1990, Handy and Kennelly, 1983, Kennelly and Fenton, 1982). Research work utilizing increasing inclusion levels of canola seed in the diet (Khorasani and Kennelly, 1996, Khorasani et al., 1991) also observed an increase in this ratio in milk fat.

4.5.2.6. Free Fatty Acids

The release of FFA is the result of lipolysis and fatty acid re-esterification (Chilliard, 1993). Jensen et al. (1991) states that in freshly drawn and extracted milk, only traces of FFA will be detected. These authors report that lipolysis will not change the total fatty acid composition of milk unless some of the volatile short-chain fatty acids are lost, however, lipolysis will alter the amounts of FFA. Free fatty acids are considered to be a minor component of milk fat (Brink and Lofgren, 1982). In general, the formaldehyde treated canola seed fed cows did present the lowest FFA in milk. As indicated FFA are the result of lipolysis and re-esterification which could suggest that these two processes were reduced when formaldehyde treated canola seed was included in the diet. The heat treated canola seed fed cows exhibited the greatest content of FFA in milk fat for the dietary treatments. This would suggest that lipolysis and re-esterification of fatty acids in the rumen was altered to a greater extent. Formaldehyde treatment of canola seed, therefore, may have reduced the effect of free fatty acid production in the rumen and its subsequent detection in milk.

4.5.3. Milk Protein Fractional Composition

Thomas (1984) states that more than 94% of the crude protein (N x 6.38) in milk is present as true protein and the remainder is NPN compounds. The percentage of protein in fluid milk is approximately 3.5% and approximately 80% of this is casein with the remainder

being whey proteins (Thomas, 1984, Brink and Lofgren, 1982). Thomas (1984) indicates that the proportions of protein vary and can be influenced by breed, individual cow characteristics, lactation number, stage of lactation and present and previous mastitis infections.

The fractional composition of milk protein obtained from the dietary treatments was illustrated in Table 4.9. As indicated by the protein yield and percentage (Table 4.1), the addition of canola seed to the early-lactation diet did not result in significant differences or a significant depression in protein content. No significant differences for protein yield, percentage or CP expressed as a percentage of milk were observed for the current study. Khorasani et al. (1991) and Drackley and Elliott (1993) both reported that inclusion levels of protected fat sources higher than 4% (dietary DM) resulted in a decline in CP. The true protein and casein protein fractions were significantly altered by dietary fat supplementation. Cows fed the untreated diet had elevated true protein and casein fractions compared to the cows fed the formaldehyde treated canola diet which had the lowest percentage. Jenkins and Jenny (1992) state that not all fat sources have equivalent effects on protein fractions of milk. No significant effects were observed for NPN and whey protein fractions in milk with the different dietary treatments. Khorasani et al. (1991), Jenkins and Jenny (1992) and Drackley and Elliott (1993) found that the NPN fraction was not significantly different among dietary treatments when supplemental fat was added.

True protein, casein protein, NPN and whey protein expressed as a percentage of CP were not influenced by dietary treatment. Other researchers also observed no treatment effects with the addition of supplemental fat (Khorasani et al., 1991, Drackley and Elliott, 1993, DePeters et al., 1985). DePeters et al. (1989) states there has been little work appraising the effect of diet on the casein concentration in milk and more information is needed on the distribution of N in milk. Rowland (1938) indicates that normal milk contains approximately 77 to 81% of the total milk N as casein N. This is in agreement with the current percentages obtained, however, changes in total protein of milk are quite small because a decrease in the casein fraction is frequently accompanied by an increase in whey protein.

Milk proteins are classified as the casein fraction or the whey or non-casein fraction.

Swaisgood (1982) provides a brief description of some of the casein and whey proteins found in milk protein and indicates their approximate concentrations. The beta casein fraction was significantly affected due to the addition of canola seed. The levels of alpha and beta casein and lactoglobulin were within the ranges suggested by Swaisgood (1982). The value suggested by Swaisgood (1982) for lactalbumin (1-1.5 mg/ml) was slightly lower than what was found for the current study. DePeters et al. (1989) found that whey protein was reduced with the addition of added fat to the diet possibly reflecting an inhibition of protein synthesis within the mammary gland for lactalbumin and lactoglobulin. The values obtained for the fractionation of the casein and whey proteins obtained are relatively small (mg/ml) and were slightly affected by the fat source in the diet. Ashes et al. (1995), however, states milk protein components which were fractionated from cows fed protected canola seed supplements found the proportions of casein, lactoglobulin and other milk proteins were similar to those found in the control milk. This was observed for the current study although some protein fractions were influenced by the addition of the canola seed.

The fractional composition of milk protein can be affected by the addition of dietary fat but the extent of its effect is variable. The dietary treatments in this experiment did exhibit different rates of disappearance and degradation as illustrated by the *in situ* nylon bag study and CP digestibility was not influenced by dietary treatment as was illustrated in Table 2.6. Protein degradation in the rumen, therefore, may not have influenced milk protein composition.

4.5.4. Mineral Composition in Milk

Minerals are generally classified into two specific groups, (1) macro-minerals or major minerals and (2) micro-minerals or trace minerals. The macro-minerals include Ca, Mg, Cl, P, K, Na and S. Brink and Lofgren (1982) state there are 103 known trace elements, however, only 17 exhibit biological function in animals. The trace elements which are evaluated in human nutrition include Cu, Fe, Mn, Zn, Cr, F, I, Mo and Se. The trace elements in milk are derived from the feedstuffs consumed by the dairy cow, water or the environment. According to Brink and Lofgren (1982), the concentrations of these minerals are approximate

as values in the literature are highly variable due to the stage of lactation, season, milk yield, amount of trace element in the cow's diet, handling of the milk following pasteurization, storage conditions and methods and accuracy of analysis.

The mineral content of milk was studied to determine if there was any effect on the mineral content due to the addition of canola seed in the diet. The mineral content of the dietary treatments was not determined but were assumed to be similar. The ration formulation did not adjust for Ca content, therefore, no supplemental Ca was added to either the control or canola seed treatments. Palmquist and Jenkins (1980) suggest that addition of unprotected fat and fatty acids to the diet may influence Ca absorption due to soap formation of Ca. It is postulated that Ca improves fibre digestibility of high fat rations by forming insoluble soaps which remove the fatty acids from solution so they are no longer available to bind to rumen microorganisms (Palmquist and Jenkins, 1980). From the data compiled, no significant differences were found for the major or trace minerals except for Fe and B. The significant differences in Fe and B could have been due to the dietary treatment, however, this can not be substantiated since mineral content of the diet was not determined. As there were no major significant differences in mineral composition in milk, it can be speculated that the canola seed added to the diet did not influence the mineral content of the milk. The mineral composition of the feed, however, should be analyzed to determine if there was any influence of the diet itself on the mineral composition of the milk. The mineral composition for the milk samples was within the range of approximate values stated by Brink and Lofgren (1982).

4.6 Conclusion

Daily milk yield, protein yield and percentage, fat yield, lactose yield and percentage were not affected by supplementing the diet with canola seed, however, fat percentage in milk was significantly lower for cows fed the untreated canola seed diet. The protein fractions determined were generally not influenced by the additional fat source, however, significant differences were observed when true protein and casein protein were expressed as a percentage of milk. The fractional composition of protein expressed as a percentage of CP was not significantly different for the dietary treatments.

Dietary manipulation of milk fat in the lactating dairy cows by supplementing the diet with protected and unprotected canola seed resulted in alterations in milk fatty acid composition. Supplementing the diet with either unprotected or protected canola seed caused a decline in short- and medium-chain fatty acids and an increase in the content of long-chain fatty acids due to reduced de novo synthesis in the mammary gland. The magnitude of the changes in fatty acids in milk, however, depends upon the type of added fat to the diet. Substantial increases in the polyunsaturation of milk fat resulted from the inclusion of formaldehyde treated canola seed in comparison to the untreated and heat treated canola seed diets. Cows fed the formaldehyde treated canola seed diet in comparison to the heat treated diet resulted in a greater reduction in the saturated fatty acids and increases in the monounsaturated and polyunsaturated fatty acids in milk fat

Not all of the components observed for milk fatty acid composition resulted in significant differences between the dietary treatments. The impact of protected canola seed inclusion in the lactating dairy cow diet on milk fatty acid composition suggests that treated and untreated canola seed can be used to alter milk fat composition, however, unprotected canola seed is less effective. Whether or not protected canola seed will be utilized on the farm instead of unprotected canola seed will depend on such factors as the cost of production of the protected supplement, consumer acceptance of milk produced from formaldehyde treated canola seed, producer reactions, the potential market for milk produced from protected fat sources and the interest of how effective the protected supplement can affect milk fatty acid composition.

It can be stated that the hypothesis of this experiment was achieved by the data obtained. Heat treatment and formaldehyde treatment of canola seed did increase the supply of unsaturated fatty acids (monounsaturated and polyunsaturated fatty acids) to the intestine and, thus, provided an efficacious method of altering the fatty acid composition of milk. Of the two protected treatments utilized, the formaldehyde treatment provided the best protection against ruminal degradation as judged by the reduced ruminal disappearance and degradation values obtained in the nylon bag study as well as its digestibility characteristics. As a result, there was a greater concentration of unsaturated fatty acids and reduced

concentration of saturated fatty acids in milk fat. The biggest question, however, is what should the level of formaldehyde treated canola seed in the diet be in order to get the beneficial changes in milk fat composition without a negative effect on rumen fermentation and cow performance. Further research on the inclusion levels of formaldehyde treated canola seed, therefore, should be considered. From a human health perspective, utilization of protected fat sources has resulted in a decrease in the saturated fatty acids with subsequent increases in mono and polyunsaturated fatty acids in milk.

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CHAPTER 5

General Discussion and Conclusions

5.1. Introduction

The characteristic feature of bovine milk is the high proportion of short- and medium-chain fatty acids and the low proportion of polyunsaturated fatty acids (Brumby and Welch, 1978). Typical milk fat is 70% saturated, 25% monounsaturated and 5% polyunsaturated fatty acids (Grummer, 1991). A question which is addressed through research work is "Why alter milk fat composition?" Interest in altering milk fat composition has resulted from consumers being concerned about their intake of fat, cholesterol, calories and the nutritional value of food. The medical profession has also linked saturated fat to cardiovascular disease. Health concerns have resulted in a decline in milk fat consumption due to its high level of saturated fat (Jensen et al., 1991) and consumers have shifted from whole milk to 2%, 1% or skim milk.

In order to supply unsaturated fatty acids to the consumer, the diet of the dairy cows can be manipulated. Fatty acids in milk are derived from two major sources (1) acetate and β-hydroxybutyrate and (2) circulating blood lipids derived from carbohydrates and fat in the diet. Adipose tissue can also be mobilized as a source of fatty acids in milk. Milk fat is estimated to be made up of 50% of the fatty acids from de novo synthesis, 40-45% from dietary sources and 10% from adipose tissue mobilization (Palmquist and Jenkins, 1980). Acetate and β-hydroxybutyrate are the major carbon sources for de novo synthesis which arises from rumen fermentation. Short-chain fatty acids ($C_{4:0}$ - $C_{10:0}$) are synthesized primarily from de novo synthesis in the mammary gland primarily from acetate and β-hydroxybutyrate. Long-chain fatty acids, C_{18} and greater are derived from circulating triglycerides in blood plasma from dietary lipids and adipose tissue mobilization. Medium- chain fatty acids ($C_{12:0}$ - $C_{16:0}$) are derived from both routes.

In ruminant animals, fatty acid biosynthesis is influenced by the conditions of the rumen as this will determine the microbial population found in the rumen (Palmquist and

Jenkins, 1980). In the rumen, biohydrogenation affects the fatty acids reaching the mammary gland. Long-chain fatty acids of dietary origin are hydrogenated to saturated and monounsaturated fatty acids, therefore, polyunsaturated fatty acids (ie: $C_{18:2}$, $C_{18:3}$) are absent since they are hydrogenated to $C_{18:0}$ or a number or geometrical or positional isomers of $C_{18:0}$. This results in milk fat being relatively saturated compared to the diet. To prevent biohydrogenation of dietary fat in the rumen, unsaturated fatty acids must be protected from rumen biohydrogenation. This will result in polyunsaturated fatty acids being digested and absorbed postruminally and can be incorporated in milk fat.

De novo synthesis in the mammary gland of short- and medium-chain fatty acids and dietary and microbial long-chain fatty acids presented to the mammary gland can be altered by dietary manipulation (Kennelly and Khorasani, 1992). The ability of the mammary gland to incorporate long-chain fatty acids into milk fat provides the opportunity to alter the content and type of fat present in milk fat (Kennelly, 1996a). Kennelly (1996b) indicates that dietary manipulation causing an increase in $C_{18:0}$ and $C_{18:1}$ fatty acids at the expense of $C_{16:0}$ and $C_{14:0}$ is considered to be desirable from a human health and medical perspective. Two current methods used to reduce rumen biohydrogenation and alter milk fatty acid composition are heat or chemical (formaldehyde) treatment. Heat treatment decreases the solubility of proteins by creating cross linkages both within and among peptide chains and to carbohydrates, therefore, lowering susceptibility to ruminal degradation (Deacon et al., 1988). Formaldehyde reacts with the terminal amino acid groups of protein and epsilon amino groups of lysine, resulting in methylene bridge formation and rendering protein more resistant to microbial degradation (Weakley et al., 1983).

The overall hypothesis of the current study was that heat or chemical treatment of canola seed would increase the supply of unsaturated fatty acids to the intestine and, thus, provide an efficacious method of altering milk fatty acid composition. The overall objectives of the study to determine if the hypothesis was correct was to use an *in situ* nylon bag study for the determination of disappearance and degradation of canola seed and an animal trial to study the effect of dietary fat on ruminal and whole tract digestion and milk yield and composition.

5.2. Overall Experimental Conclusions and Implications

The nylon bag technique is a quick, effective and widely accepted method to determine the rate and extent of ruminal digestion of a feedstuff. Results have shown that the formaldehyde treatment of canola seed decreased the rate of degradation of its protein in the rumen and increased its undegradable insoluble protein (UIP) content. Changes in rumen degradation characteristics of canola seed related to heat treatment, however, were small. The effect of added dietary fat (at 2.1%) on rumen digestion and whole tract digestibility of nutrients was negligible. The results also indicated that preparation of formaldehyde treated canola seed as a protection method is feasible. The heat treatment utilized in the current study alone can not be recommended, however, Jet-Sploding techniques have proven to be successful (Khorasani and Kennelly, 1996, Khorasani et al., 1991). The benefits of greater dietary energy density associated with increasing dietary fat content at a low level (2%) on animal performance was minimal.

The impact of formaldehyde treated canola seed on milk fat fatty acid composition suggests that formaldehyde treatment has a positive effect on ruminal escape of fat in canola seed. The effect of heat treatment of canola seed, however, was minimal on protecting fat from rumen bacterial degradation of fatty acids. This current experiment demonstrated that treating canola seed with formaldehyde is capable of protecting the canola seed protein from rumen degradation, thus, increasing the bypass protein supply of the diet. The level of added fat in this study was low, therefore, the effect of added dietary fat on rumen fermentation and animal performance was not substantial. The results of the current study indicate that formaldehyde treatment of canola seed is a feasible procedure for altering milk fatty acids resulting in a decline in saturated fatty acids and a subsequent increase in unsaturated fatty acids. This result was also found in previous work of Ashes et al. (1995, 1992). A high concentration of $C_{16:0}$ and $C_{14:0}$ in milk continues to be a concern to the medical field and the medical community (Kennelly, 1996b). Kennelly (1996b) reports that dietary manipulation which results in a decline in $C_{16:0}$ and $C_{14:0}$ in milk fat and results in an increase in $C_{18:0}$ and $C_{18:1}$ fatty acids in milk is considered desirable from a human health perspective. An increase in $C_{18:0}$ and $C_{18:1}$ fatty acids was observed in this study with a decline in the levels of $C_{16:0}$ and

$C_{14:0}$

The extent to which dietary fat will alter the fatty acid composition as well as the overall composition of milk depends on a number of factors. These factors include: (1) the degree of ruminal hydrogenation, (2) composition of the non-lipid component of the diet, (3) influence of the lipid source on microbial fatty acid synthesis and de novo synthesis of fatty acids in the mammary gland, (4) influence of the lipid source on the rumen environment, (5) stage of lactation, (6) intestinal and mammary desaturase activity, (7) fat source and inclusion level or (8) the protection method utilized. Kennelly (1996b) states that the ability of the mammary gland to secrete fatty acids in milk is not a limiting factor in feeding strategies designed to alter milk composition since previous research in Australia has produced milk containing 30 and 20% $C_{18:2}$ and $C_{18:3}$, respectively, when cows were fed a protected lipid supplement containing linseed and safflower oils (McDonald and Scott, 1977). Grummer (1991) comments that the degree of success in altering milk fatty acid composition by feeding formaldehyde or heat treated supplements has been inconsistent which reflects the variability in the degree of protection achieved among laboratories.

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Appendix 2.1 Extraction Method for Determination of Lipid Content

1. 0.2 g of sample was used for all sample times except for the 24 hour time in which 0.25 g was used.
2. Folch II solution (chloroform: methanol: water (3:47:48)) was not used.

Procedure:

1. For times 0.1, 2, 4, 8 and 16 hour samples 0.2 g and for the 24 h sample 0.25 g were weighed into 50 ml Teflon lined screw cap tubes. (In duplicate)
2. 20 ml of Folch I solution (chloroform: methanol (2:1 v/v)) was added.
3. Homogenized samples with a polytron for approximately 30 sec each.
4. Rinse polytron head with 10 ml of Folch I solution to remove any remaining particles.
5. Allow samples to sit for 2 h.
6. After 2 h, filter samples through glass wool into 100 ml graduated cylinders (with glass stoppers).
7. Rinse tubes and filter paper with 10 ml of Folch I solution.
8. Add 10 ml of 0.8% NaCl solution to the graduated cylinder, mix solution gently and leave overnight for layers to separate.
9. After the layers have separated, record volume of the lower chloroform layer and calculate the percentage of fat by the following formula:

$$\% \text{ Fat} = \frac{(\text{Vial} + \text{fat}) - \text{Vial wt.}}{\text{sample wt.}} \times \text{chloroform volume} \times 100\%$$

10. Correct fat percentage for dry matter.

Appendix 3.1. Lactic Acid Analysis Procedure.

1. Thaw frozen samples.
2. Shake well after thawing.
3. Centrifuge at 6°C for 15 min at 4,000 rpm (head size = JA-20).4. After centrifuging, add 1 ml of supernatant to screw cap test tubes (13 x 100 mm or 16 x 100 mm), 0.2 ml of internal standard (malonic acid) and 0.2 ml of 3N NaOH.
    -Internal standard: malonic acid (disodium salt) (molecular weight =148 g/mol)
        -standard composition: 0.3997 g of malonic acid in 200 ml of distilled water.
    -External standard: lactic acid (lithium salt) (molecular weight = 96.01 g/mol).
        -standard composition: 0.2162 g lactic acid per 100 ml of distilled water.
5. Samples mixed and cover with perforated parafilm and evaporate dryness in a freeze-dryer (-60°C, shelf heat 15°C).
6. Once the samples dried to complete dryness (powder dry), add 1 ml of methanoic-HCl and cap test tubes , vortexed and heated for 25 min at 110°C in a forced air oven.
7. After heating, revortex samples, allow to cool and allow precipitate settle overnight.
8. After settling, transfer supernatant to gas chromatography vials and analyze using gas chromatography.

Conditions For Gas Chromatography:
1. Instrument used to analyze the lactic acid derivative: Varian model 3400 with a megabore capillary column STABILWAX-DA (30 m, 0.5 mm I.D.).
2. Temperature program used for analysis:
    -initial temperature of 80°C for 1 min
    -final temperature of 180°C for 8 min
    -column rate of 20°C/minute
    -initial auxiliary temperature (autosampler injector port) of 180°C
    -detector temperature of 190°C.
3. Autosampler wash bottle filled with methanol.
4. Volume of sample injected was 0.5 μl and the solvent plug size was <0.3 μl.
5. Splitter flow rate approximately 40 ml/minute.
6. Column pressure approximately 7 psig.

With this temperature setting and a megabore capillary column, the lactic acid derivative eluted at approximately 3.65 min and the internal standard at approximately 5.3 min. The length of one run was 14 min. Calculations were done for impurities in the internal and external standards for malonic acid (disodium salt) and lactic acid (lithium salt), respectively. Automatic calculations for response factors (using the absolute amount of internal standard in the tube in mg) calculated using Shimadzu Ezchrom 3.2 version data system (Shimadzu Scientific Instruments, Inc., 7102 Riverwood Drive, Columbia, MD, 21046). Reprocessing of the samples gave the lactic acid derivative concentration in mg/ml. For quality control, control rumen fluid was carried with every sample preparation and each run of samples to ensure consistency in gas chromatography performance.

Appendix 3.2. Volatile Fatty Acid Analysis Procedure.

1. Thaw frozen rumen fluid samples.
2. Centrifuge at 4°C for 15 min at 4, 000 rpm (head size= JA-20).3. After centrifuging, transfer 1 ml of supernatant to gas chromatography vials and 200 μl of internal standard (4-methyl valeric acid).
4. Cap vials with a rubber septum, mix and ready for gas chromatography analysis.
5. External VFA standards prepared for the determination of the response factor of each VFA.
    -Composition in grams (in 100 ml of distilled water) of the external standard:
        -0.3017g acetic acid, 0.1292 g propionic acid, 0.0096 g isobutyric acid, 0.08 g butyric acid, 0.0142 g valeric acid, 0.0187 g isovaleric acid, 0.0099 g caproic acid.
6. External standard mixture used for analysis was 0.2 ml of 25% ortho-phosphoric acid, 0.80 ml external VFA standard and 0.2 ml of the internal standard.
7. Control rumen fluid was prepared by the addition of 0.80 ml of rumen fluid, 0.2 ml of internal standard and 0.2 ml of phosphoric acid to ensure quality control and consistency in gas chromatography performance.

Conditions for Gas Chromatography:
1. Instrument used to analyze the lactic acid derivative: Varian model 3400 with a megabore capillary column STABILWAX-DA (30 m, 0.5 mm I.D.).
2. Temperature program:
    -injector temperature of 170°C
    -detector temperature of 200°C
    -initial temperature of 120°C for 1 min
    -column rate of 10°C /minute, final temperature of 180°C for 2 min.
3. Flow rate of helium was 30 ml/min and a chart speed of 0.5 cm/min.
4. Response factor for each VFA was calculated using the Shimadzu Ezchrom 3.2 version data system (Shimadzu Scientific Instruments, Inc., 7102 Riverwood Drive, Columbia, MD, 21046). Reprocessing of the samples gave the concentration of the VFA in mg/ml. For quality control, control rumen fluid was carried with every sample preparation and with each run of samples to ensure consistency in gas chromatography performance.

Appendix 3.3. Ammonia Nitrogen Analysis Procedure

Ammonia Nitrogen Analysis for Rumen Fluid:

1. Thaw rumen fluid.
2. Shake and centrifuge at 5°C for 15 min at 4,000 rpm (head size= JA-20).
3. After centrifuging, 20 μl of supernatant pipetted in duplicate into 16 x 100 mm borosilicate disposable (glass) test tubes and covered with parafilm.
4. Add reagents to standards and samples and put into a dark cupboard at room temperature for 1 h for color development.

Reagents:

1. Sodium phenate (12.5 g phenol + 6.2 g NaOH in 500 ml volumetric flask made to volume with deionized water).
2. Sodium Nitropurside- stock solution (1%) - 1 g/100 ml water
   - working solution (0.01%)- 5 ml stock diluted up to 500 ml (volumetric flask) with deionized water.
3. Sodium Hypochlorite - 0.02N, 15 ml NaOCl (4-6%) diluted to 500 ml with deionized water, pH = 12 with 50% NaOH.

Conditions for Spectrophotometer:

1. Absorbance read on a dipping probe at 600 nm (Spectronic 3000 Array, The Milton Ray Company, V1.14 Analytical Products Division)using deionized water to zero spectrophotometer

-pump speed: 80, pump time:6, purge time: 3

2. Software: RapidScan Rev.2.1 (1991, The Milton Ray Company) and standard curves and absorbance/transmittance were the applications utilized. A standard curve was used to determine the absorbance of the samples being analyzed and the amount of ammonia N in the individual samples. The concentration of ammonia N in rumen fluid was extrapolated directly from the spectrophotometer readings.

Ammonia Nitrogen Analysis for Duodenal Digesta:

1. Thaw and shake duodenal digesta.
2. Approximately 5 ml of fluid transferred to 16 x 100 mm glass tubes, centrifuged for 10 min (1,000 rpm, 5°C, head size = JA-20).
3. After centrifuging, 40 μl aliquots of supernatant pipetted into 16 x 100 mm borosilicate glass test tubes in duplicate and covered with parafilm.
4. The same color development procedure was used as for the rumen fluid samples.
5. The calculation of ammonia N for duodenal digesta is as follows:

Dry sample = DM at 110°C x DM at 60°C x (1/100)
Concentration mg N = average ug/tube x (1000 ÷ volume added) x (1/100)
Total N = concentration mg N x (1000 - dry sample)
gram NH_3/ kg sample = Total N ÷ dry sample.

Appendix 3.4. Chromium Marker Preparation.

Forage Preparation:

1. Grind 8.5 kg of alfalfa hay through a 5 mm screen (Fritz® Mill Model D Serial #8972 Comminutor by The Fritzpatrick Company).
2. Place 8 kg ground alfalfa hay into a large metal garbage pail with a hose outlet secured at the bottom.
3. Fill container with hot water and cover with metal screen mesh and tie securely. Run hot water through the hay for approximately 2 h followed by cold water overnight.
4. After overnight washing of the forage, turn water off and invert pail and allow to drain for approximately 2 h.

Cr-mordant Forage Preparation:

1. Dissolve 2.85 kg of sodium dichromate in a container of hot water.
2. Add hot water to cover the forage and pour the sodium dichromate solution into the forage.
3. Stir mixture and fill container with hot water to approximately 15 cm below the top.
4. Place metal garbage can into a plastic container, cover with the garbage can lid and place into a 105°C forced air oven for 24 h.
5. After 24 h, shut oven off and allow the mixture to cool and remove metal garbage pail from plastic container.
6. Wash mixture with cold water until waste solution is clear and collect waste solution in waste disposal containers.
7. Reconnect hose to the hose outlet and wash forage mixture for 24 h with cold water.
8. After 24 h, invert the garbage pail and drain for approximately 2 h.
9. Dissolve 4 kg of ascorbic acid in a container of cold water.
10. Fill the metal garbage pail with cold water to approximately half full and add the ascorbic acid solution.
11. Stir mixture on and off for 1 h.
12. Reconnect the hose to hose outlet and wash alfalfa mixture with cold water for 24 h.
13. After 24 h, turn water off and allow to drain for approximately 2 h.
14. Remove forage from garbage pail and place onto cheesecloth (2 layers) covered shelves and dry at 60°C in a forced air oven until complete dryness.
15. Following drying, allow Cr-mordant forage to air equilibrate and grind to pass a 2 mm screen (Fritz® Mill Model D Serial #8972 Comminutor by The Fritzpatrick Company).

Appendix 3.5. Determination of Solid Phase Digestibility of Duodenal Digesta.

1. Weigh 500 mg of digesta and 50 mg of Cr-mordanted fibre in duplicate into borosilicate glass test tubes with Teflon lined screw caps, Kimax brand.
2. Soak in 30 ml of 4M nitric acid (HNO_3) for 4 h. Tightly cap the test tubes with Teflon lined screw caps, swirl and allow to digest for 12 h in a water bath at 75°C. (Continue to add additional water to water bath as water will evaporate).
3. After digestion, remove samples from water bath and allow samples to cool.
4. Wipe any excess material on the outside of the tubes and reweigh.
5. Mixed material thoroughly and filter through filter paper into 16 x 100 mm borosilicate tubes.

Standard Solution Preparation:
1. Standard solutions prepared using chromium reference solution 1000 ppm Fisher (#SO-C-192) to create a standard curve of 2 ppm, 4 ppm, 6 ppm and 8 ppm.

Absorbancies of the Solutions:
1. Standards and digesta sample absorbencies read using a Perkin Elmer 4000 (PE 4000) atomic absorption spectrophotometer.

-Conditions of atomic absorption spectrophotometer:

- a hollow cathode lamp with compressed air as an oxidant and acetylene as fuel
- a Cr lamp with a lamp current of approximately two-thirds of the maximum current
- a wavelength of 357.9, slit at 0.7H, time interval at 1, air pressure of 50 psi and acetylene pressure of 15 psi.

The amount of chromium was determined by the following calculation:

$$\text{Cr ppm} = \frac{\text{(absorbancy-intercept)}}{\text{slope}} \times \text{(postdigestion wt - predigestion weight)/sample wt/DM}$$

Appendix 3.6. Isolation of Rumen Bacteria.

1. Collection of rumen fluid was via the rumen cannula using a metal strainer connected to plastic tubing attached to a stopper on a glass flask. Suction was created by attaching another piece of plastic tubing attached to a pump connected to the glass flask.
2. After collecting rumen fluid into the flask (1L), transfer fluid to plastic nalgene containers filled with carbon dioxide.
3. Place rumen fluid samples on ice.
4. In the laboratory, samples filtered under carbon dioxide through cheese cloth to remove any solid particles into a plastic nalgene bottle.
5. Centrifuge samples for 15 min (3,200 rpm, 15°C) and the liquid phase was poured into containers and the supernatant recentrifuged.
6. The supernatant liquid was first centrifuged at 11,000 rpm for 20 min (head size = JA-14) and then the precipitate was resuspended with 0.9% saline solution and recentrifuged at 11,000 rpm for 20 min (head size = JA-14).
7. Pour off supernatant and transfer the precipitate to a loaf pan and freeze at -30°C until further analysis.
8. All the bacterial samples were pooled for each period.
9. The bacterial samples were freeze dried at -60°C until complete dryness.
10. After freeze drying, samples were transferred to plastic nalgene containers for storage and further analysis for DAPA, N, DM, OM and CP.

Appendix 4.1. Gas Chromatography Conditions for Fatty Acid Analysis in Milk.

1. Instrument:
   -Varian model 3600 with a fame ionization detector
2. Column used was a BPX70 column (25 m, 0.22 mm I.D., 0.25 micron film) (SJE Australia Pty. Ltd., Victoria, Australia)
3. Column oven temperature was held 0.05 min at 60°C, then programmed at a rate of 25°C/min to a temperature of 170°C, held for 1 min, programmed at a rate of 2°C/min to a temperature of 180°C, held for 2 min, programmed at a rate of 10°C/min to a final temperature of 220°C.
4. Column injector with a septum programmable injector (SPI) was 70°C at 0 min and programmed at a rate of 150°C/min to a final temperature of 230°C and held for 16 min.
5. Injector and detector temperatures were 230°C.
6. The end time of the run was 17.45 min.
7. The sample volume taken was 0.5 μl and the solvent plug size was 0.3 μl.
8. The flow rate of the helium as a make up gas was 30 ml/min, helium as a carrier gas at approximately 15 psi pressure, hydrogen at 30 ml/min and air at 300 ml/min.
9. The peak areas for each fatty acid were processed with Shimadzu Ezchrom 2.1 version data system (Shimadzu Scientific Instruments, Inc., 7102 Riverwood Drive, Columbia MD, 21046) and were expressed as percentages of total fatty acids detected. Identification of the fatty acid peaks was based on the retention times of standard methyl esters of individual fatty acids.

Appendix 4.2. Procedure for Determining Free Fatty Acid Content in Milk.

1. Pipette 10 ml of milk into 50 ml glass test tubes with Teflon lined screw caps. and
2. Add an internal standard (0.01 ml containing 0.5 mg/ml each of heptadecanoic acid and tridecanioc acid in heptane) and mix.
3. To extract the FFA, add 15 ml of cold ether:heptane (1:1, v/v) and hand shake for 30 sec.
4. Centrifuge samples at 2,500 rpm for 2 min.
5. Transfer upper solvent layer into a 50 ml glass test tube with a Teflon lined screw cap.
6. Add 1 ml of sulfuric acid (2.5 mol/L) to the sample and mix.
7. Add 15 ml of cold ether:heptane and mix by repeated inversion.
8. Centrifuge samples at 2,500 rpm for 2 min.
9. Transfer the upper solvent layer to 50 ml glass test tubes.
10. Add cold ether:heptane (1:1, v/v) and centrifuge twice and 10 ml of cold ether:heptane was used.
11. Combine solvent layers in a second 50 ml glass tube test tube.
12. To isolate the FFA an anion-exchange column was prepared by placing 0.2% of aminopropyl resin in a 3 ml syringe with stainless steel frits (Supelco Ltd., Mississauga, Ontario, Canada). Condition columns with 10 ml of heptane. The lipid extract was applied to the column at a flow rate of approximately 1 ml/min. Neutral lipids were eluted from the column with 5 ml of chloroform: 2-propanol (2:1, v/v). Free fatty acids were eluted with 10 ml glass test tubes with Teflon lined screw caps with 3 ml of ether containing 2% formic acid. One millilitre was placed on the column and flow was stopped when the ether extract reached the top of the resin. This was allowed to sit for 30 sec and repeated twice. The recovery standard (0.1 ml containing 0.5 mg/ml of undecanoic acid in heptane) was added, mixed and 1.3 ml of this sample was added to the 1.5 ml chromatography vial. Determination of the amount of FFA in the milk sample was based upon the amount of internal standard added to the sample.

Appendix 4.3. Procedure for Determining the Content of Casein Proteins in Milk.

1. Precipitating casein proteins (in 2.5 ml milk) with a mixture of approximately 2 ml of water, 10% acetic acid (approximately 10:1, milk:10% acetic acid) and sodium acetate (0.1N) (approximately 12.5:1, milk:0.1N sodium acetate).
2. To isolate the casein proteins, centrifuge samples at 3,000 rpm for 15 min.
3. Remove aqueous layer using a pasteur pipette and leaving the casein precipitate and fat layer behind.
4. Retain aqueous layer for determination of whey proteins in milk.
5. Add 2 ml of water to the casein precipitate, frozen at -20°C and freeze dried (-60°C, shelf heat 15°C).
6. Prepare standards by weighing out increasing amounts of casein of determined composition (approximately 30 to 75 mg) into test tubes.
7. Analyze standards and casein samples by reverse-phase high performance liquid chromatography (HPLC).
8. Add approximately 5 ml of 0.02M of 1,3-bis[tris(hydroxymethyl)-methlyamino] propane (pH7) containing 4M urea and 0.3% of 2 mercaptoethanol (TRIS-BIS buffer) to the standards and the casein samples.
9. Incubate standards and the casein samples for 1 h (37°C) and mix occasionally.
10. Decant one half of each sample into disposable culture tubes and centrifuge at 3,000 rpm for 15 min.
11. Retain the portion remaining as a backup sample.
12. Suction the upper fat layer off and pipette approximately 0.2 ml of sample or 1.5 ml of standard into 1.5 ml HPLC vials.
13. Add approximately 1 ml of 6M urea containing approximately 0.2% trifluoroacetic acid to the HPLC vial and mix well.

Conditions of the HPLC:
1. Three buffer solutions:
   - -Buffer A : water:trifluoroacetic acid (1000:2)
   - -Buffer B : water:acetonitrile:trifluoroacetic acid (500:500:2)
   - -Buffer C : acetonitrile:trifluoroacetic acid (1000:2).
2. HPLC column used: Vydac C_{18} reverse-phase column (Separations Group, Hesperia, CA).

3. Total run time:65 min, flow rate of 1 ml/minute and an ultraviolet detector with a wavelength of 220 nm to determine the casein proteins.
4. To determine the content of casein proteins in milk, a standard curve was calculated from the amount of individual casein in the standard and their peak area responses. The amount of individual casein in each milk samples was calculated by extrapolation of their peak area responses to amounts using the standard curve.

Appendix 4.4. Determination of the Content of Whey Proteins in Milk.

1. The same procedure used to isolate the casein proteins was used to isolate whey proteins up to the casein isolation step.
2. The aqueous layer retained during casein isolation was centrifuged at 3,000 rpm for 15 min and any residue was siphoned off the top of the aqueous layer.
3. Transfer approximately 1.3 ml of the aqueous layer into a 1.5 ml HPLC vial.
4. Standards of similar composition to that of the aqueous layer (milk serum) and containing increasing amounts of whey protein of known contents were run with the samples to generate a standard curve.
5. Buffer preparation involved preparation of a solution of 1% formic acid (v/v) and 10% sodium chloride (w/v) in HPLC water:
   -Buffer A: water:acetonitrile:sodium chloride:formic acid (900:100:10:1)
   -Buffer B: water:acetonitrile:sodium chloride:formic acid (400:600:10:1).
6. Analysis of the whey proteins was by using HPLC:
   -Vydac C_4 or C_{18} reverse-phase column (Separations Group, Hesperia, CA).
   -total run time was approximately 25 min, a flow rate of 1 ml/min and an ultraviolet detector at a wavelength of 280 nm for the determination of whey proteins.
7. The calculation to determine the content of whey proteins in milk involved a standard curve being calculated from the amount of individual whey proteins in the standard and their peak area response. The amounts of individual whey proteins in each milk samples were calculated by extrapolation of their peak responses to the amounts using the standard curve.

Appendix 4.5 Transfer Efficiencies for Fatty Acids in Milk.

ASSUMPTIONS:

The transfer efficiency was calculated as the change in yield of individual fatty acids in cows fed the test diets (untreated, formaldehyde treated and heat treated canola seed) compared to those fed the control diet, taking into account the intake of fatty acids for animals fed the test diets. The fatty acid composition of the TMR was not determined.

DETAILEDCALCULATION OF TRANSFER EFFICIENCY OF FATTY ACIDS (FA)

Equation 1: FA from feed absorbed (FAf)

FAf (g/d) = DMI × IL × FS × FAS × DIG × 1000

where DMI = dry matter intake (kg/d)
IL = inclusion level of 5%
FS = fat percentage in seed
FAS = fatty acid percentage in seed
DIG = digestibility of fat in whole tract (%)

Equation 2: FA in test milk (FAt)

FAt (g/d) = FYt × FA

where FYT = fat yield in milk (g/d) for cows fed the test diet
FA = fatty acid percentage in milk

Equation 3: FA in control milk (FAc)

FAc (g/d) = FYc × FA

where FYc = fat yield in milk (g/d) for cows fed the control diet
FA = fatty acid percentage in milk

Equation 4: Transfer Efficiency (TE) (%)

$$\text{TE (\%)} = \frac{\text{Equation 2 - Equation 3}}{\text{Equation 1}} \times 100$$

VALUES USED FOR CALUCATION OF TRANSFER EFFICIENCY:

Feed intakes (kg/d) and fat digestibility (whole tract)(%).

	Diet			
Parameter	Control	Untreated	Formaldehyde treated	Heat treated
Feed intake, kg/d	20.29	22.76	20.67	20.33
Fat digestibility, %	63.31	68.87	72.63	64.10

Amount of fatty acids in canola seed (%) (From Table 2.1):

	Diet		
Fatty Acid (%)	Untreated	Formaldehyde treated	Heat treated
$C_{18:0}$	1.9	2.9	1.9
$C_{18:1}$	58.7	61.8	57.8
$C_{18:2}$	20.9	18.2	21.4
$C_{18:3}$	10.9	8.7	11.0
Fat, %	48.9	33.0	44.9

Fatty acids in milk (%) and fat yield (kg/day) (From Table 4.1 and Table 4.4)

	Diet			
Fatty Acid	Control	Untreated	Formaldehyde treated	Heat treated
$C_{18:0}$	9.92	13.14	11.10	11.73
$C_{18:1}$	19.77	22.57	23.98	22.70
$C_{18:2}$	1.96	1.98	3.45	2.05
$C_{18:3}$	0.51	0.57	1.14	0.57
Fat yield (kg/day)	1.28 (1280 g/day)	1.14 (1140 g/day)	1.30 (1300 g/day)	1.24 (1240 g/day)

TRANSFER EFFICIENCY:

Transfer efficiencies of C18 Fatty Acids in Milk (%)

	Diet		
Fatty Acid	Untreated	Formaldehyde treated	Heat treated
$C_{18:0}$	313.5	240.2	332.2
$C_{18:1}$	1.88	38.33	16.81
$C_{18:2}$	0	43.83	0.53
$C_{18:3}$	0	38.47	1.68

CALCUATIONS:

C18:3

Test diet: Untreated canola seed

FAf (g/d) = DMI × IL × FS × FAS × DIG × 1000 (Equation 1)

= 22.76 kg/d × 0.05× 0.109 × 0.489 × 0.6887 × 1000

= 41.77 g/d C18:3

FAt (g/d) = FYt × FA (Equation 2)

= 1140 g/d × 0.0057

= 6.50 g/d

FAc (g/d) = FYc × FA (Equation 3)

= 1280 g/d × 0.0051

= 6.53 g/d

$$\text{TE (\%)} = \frac{\text{Equation 2 - Equation 3}}{\text{Equation 1}} \times 100$$

$$= \frac{6.50 \text{ g/d} - 6.53 \text{ g/d}}{41.77 \text{ g/d}} \times 100$$

= 0% (no transfer of C18:3)

Test diet: Formaldehyde treated canola seed

FAf (g/d) = DMI × IL × FS × FAS × DIG × 1000 (Equation 1)

= 20.76 kg/d × 0.05× 0.087 × 0.33 × 0.7263 × 1000

= 21.55 g/d C18:3

FAt (g/d) = FYt × FA (Equation 2)

= 1300 g/d × 0.0057

= 14.82 g/d

FAc (g/d) = FYc × FA (Equation 3)

= 1280 g/d × 0.0114

= 6.53 g/d

$$\text{TE (\%)} = \frac{\text{Equation 2 - Equation 3}}{\text{Equation 1}} \times 100$$

$$= \frac{14.82 \text{ g/d} - 6.53 \text{ g/d}}{21.55 \text{ g/d}} \times 100$$

=38.47%

Test diet: Heat treated canola seed

FAf (g/d) = DMI × IL × FS × FAS × DIG × 1000 (Equation 1)

= 20.33 kg/d × 0.05× 0.11 × 0.449 × 0.641× 1000

= 32.18 g/d C18:3

FAt (g/d) = FYt × FA (Equation 2)

= 1240 g/d × 0.0057

= 7.07 g/d

FAc (g/d) = FYc × FA (Equation 3)

= 1280 g/d × 0.005

= 6.53 g/d

TE (%) = Equation 2 - Equation 3 × 100

Equation 1

= 7.07 g/d - 6.53 g/d × 100

32.18 g/d

= 1.68%

C18:2

Test diet: Untreated canola seed

FAf (g/d) = DMI × IL × FS × FAS × DIG × 1000 (Equation 1)

= 22.76 kg/d × 0.05× 0.209 × 0.489 × 0.6887 × 1000

= 80.10 g/d C18:2

FAt (g/d) = FYt × FA (Equation 2)

= 1140 g/d × 0.0198

= 22.57 g/d

FAc (g/d) = FYc × FA (Equation 3)

= 1280 g/d × 0.0196

=25.09 g/d

TE (%) = Equation 2 - Equation 3 × 100

Equation 1

= 22.57 g/d - 25.09 g/d × 100

80.10 g/d

= 0% (no transfer of C18:2)

Test diet: Formaldehyde treated canola seed

FAf (g/d) = DMI × IL × FS × FAS × DIG × 1000 (Equation 1)

= 20.76 kg/d × 0.05× 0.182 × 0.33 × 0.7263 × 1000
= 45.08 g/d C18:2

FAt (g/d) = FYt × FA (Equation 2)
= 1300 g/d × 0.0345
= 44.85 g/d

FAc (g/d) = FYc × FA (Equation 3)
= 1280 g/d × 0.0198
= 25.09 g/d

TE (%) = Equation 2 - Equation 3 × 100
Equation 1
= 44.85 g/d - 25.09 g/d × 100
45.08 g/d
= 43.83%

Test diet: Heat treated canola seed
FAf (g/d) = DMI × IL × FS × FAS × DIG × 1000 (Equation 1)
= 20.33 kg/d × 0.05× 0.214 × 0.449 × 0.641× 1000
= 62.61 g/d C18:2

FAt (g/d) = FYt × FA (Equation 2)
= 1240 g/d × 0.0205
= 25.42 g/d

FAc (g/d) = FYc × FA (Equation 3)
= 1280 g/d × 0.0196
= 25.09g/d

TE (%) = Equation 2 - Equation 3 × 100
Equation 1
= 25.42 g/d - 25.09 g/d × 100
32.18 g/d
= 0.53%

C18:1
Test diet: Untreated canola seed
FAf (g/d) = DMI × IL × FS × FAS × DIG × 1000 (Equation 1)
= 22.76 kg/d × 0.05× 0.589 × 0.489 × 0.6887 × 1000
= 224.97 g/d C18:1

FAt (g/d) = FYt × FA (Equation 2)
= 1140 g/d × 0.2257
= 257.30 g/d

FAc (g/d) = FYc × FA (Equation 3)
= 1280 g/d × 0.1977
=253.06 g/d

TE (%) = Equation 2 - Equation 3 × 100
Equation 1
= 257.30 g/d - 253.06 g/d × 100
224.97 g/d
= 1.88%

Test diet: Formaldehyde treated canola seed
FAf (g/d) = DMI × IL × FS × FAS × DIG × 1000 (Equation 1)
= 20.76 kg/d × 0.05× 0.618 × 0.33 × 0.7263 × 1000
= 153.08 g/d C18:1

FAt (g/d) = FYt × FA (Equation 2)
= 1300 g/d × 0.2398
= 311.74 g/d

FAc (g/d) = FYc × FA (Equation 3)
= 1280 g/d × 0.1977
= 253.06 g/d

TE (%) = Equation 2 - Equation 3 × 100
Equation 1
= 311.74 g/d - 253.06 g/d × 100
153.08 g/d
= 38.33%

Test diet: Heat treated canola seed
FAf (g/d) = DMI × IL × FS × FAS × DIG × 1000 (Equation 1)
= 20.33 kg/d × 0.05× 0.578 × 0.449 × 0.641× 1000
= 169.10 g/d C18:1

FAt (g/d) = FYt × FA (Equation 2)

= 1240 g/d × 0.2270
= 281.48 g/d

FAc (g/d) = FYc × FA (Equation 3)
= 1280 g/d × 0.1977
= 253.06g/d

TE (%) = Equation 2 - Equation 3 × 100
Equation 1
= 281.48 g/d - 253.06 g/d × 100
169.10 g/d
= 16.81%

C18:0
Test diet: Untreated canola seed
FAf (g/d) = DMI × IL × FS × FAS × DIG × 1000 (Equation 1)
= 22.76 kg/d × 0.05× 0.019 × 0.489 × 0.6887 × 1000
= 7.28 g/d C18:0

FAt (g/d) = FYt × FA (Equation 2)
= 1140 g/d × 0.1314
= 149.80 g/d

FAc (g/d) = FYc × FA (Equation 3)
= 1280 g/d × 0.0992
= 126.98 g/d

TE (%) = Equation 2 - Equation 3 × 100
Equation 1
= 149.80 g/d - 126.98 g/d × 100
7.28 g/d
= 313.46%

Test diet: Formaldehyde treated canola seed
FAf (g/d) = DMI × IL × FS × FAS × DIG × 1000 (Equation 1)
= 20.76 kg/d × 0.05× 0.029 × 0.33 × 0.7263 × 1000
= 7.21 g/d C18:0

FAt (g/d) = FYt × FA (Equation 2)
= 1300 g/d × 0.111
= 144.30 g/d

FAc (g/d) = FYc × FA (Equation 3)
= 1280 g/d × 0.0992
= 126.98 g/d

TE (%) = Equation 2 - Equation 3 × 100
Equation 1
= 144.30 g/d - 126.98 g/d × 100
7.21 g/d
= 240.22%

Test diet: Heat treated canola seed
FAf (g/d) = DMI × IL × FS × FAS × DIG × 1000 (Equation 1)
= 20.33 kg/d × 0.05× 0.019 × 0.449 × 0.641× 1000
= 5.56 g/d C18:0

FAt (g/d) = FYt × FA (Equation 2)
= 1240 g/d × 0.1173
= 145.45 g/d

FAc (g/d) = FYc × FA (Equation 3)
= 1280 g/d × 0.0992
= 126.98g/d

TE (%) = Equation 2 - Equation 3 × 100
Equation 1
= 145.45 g/d - 126.98 g/d × 100
5.56 g/d
= 332.19%

SUMMARY:

As indicated by the calculation for transfer efficiency use of unprotected canola seed in the diet results in the $C_{18:2}$ and $C_{18:3}$ being saturated in the rumen by microorganisms to either $C_{18:0}$ or $C_{18:1}$ (biohydrogenation) since there was no transfer of these fatty acids to milk fat. Heat treatment of canola seed did not result in a large transfer of $C_{18:2}$ and $C_{18:3}$ into milk fat, less than 1and 2%, respectively, also suggesting that these fatty acids were saturated to either $C_{18:0}$ or $C_{18:1}$. Protection of canola seed with formaldehyde treatment resulted in 38.5% of $C_{18:3}$ being transferred to milk, therefore, protection resulted in preventing biohydrogenation of this fatty acid. Protection of canola seed with formaldehyde treatment

also resulted in 43.8 and 38.3% of $C_{18:2}$ and $C_{18:1}$, respectively, being transferred to milk fat. Each of the canola seed treatments fed to the dairy cows had large percentages of $C_{18:0}$ transferred into milk fat suggesting that a considerable portion of the fatty acids did not escape biohydrogenation and were converted to stearic acid as illustrated in Figure 4.1.